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Studies on Human Onchocerciasis and *Simulium* in Nyanza Province, Kenya.

1. Distribution and Incidence of *O. volvulus*.

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The presence of human onchocerciasis in Kenya was apparently unsuspected until the year 1921 when Dry's observations in Lumbwa District drew attention both to the disease and to its insect vector; and although the real origin of the disease was not diagnosed at the time and the insect concerned was regarded as the probable cause of the disease rather than as a transmitter of the causal organism, Dry's description of the disease and its ecology left no doubt as to its identity which was subsequently determined by Gibbins and Loewenthal in 1933. Thereafter new records and information came to light, both published and unpublished, which established that the disease was also endemic in widely separated localities elsewhere in Nyanza Province, and from 1935 onwards the number of publications on clinical and epidemiological aspects of onchocerciasis in this region, increased notably. In this connection must be mentioned the following workers who have published papers on the subject in Kenya: Preston (1935), Harley-Mason (1939), Jobson (see in Symposium on Onchocerciasis, 1939), Hawking (1939), McMahon (1940), Harris (1940), Enzer (1942), but acknowledgment must also be made to numerous others who have contributed evidence during the course of these investigations and added to the knowledge of the subject in various ways.

In the neighbouring countries, Uganda, Sudan and Belgian Congo, the disease had been known for some time and had attracted attention by reason of its gravity in certain localities and the position in East Africa generally was summarised in the East African Medical Journal in 1939. While the gravity of the disease was well recognised it was clear that the prospects of combating it were by no means favourable in view of serious gaps in the existing knowledge. No effective therapeutic measures had yet been discovered; one of the vectors, *Simulium damnosum*, followed a breeding practice which appeared to offer insuperable difficulties to anti-larval control measures; nothing at all was known concerning the breeding habits of the other vector, *Simulium neavei*; much was yet to be learned as to the geography of the disease and its incidence in the population generally. But it was

apparent from McMahon's survey (1940) in a heavily afflicted area in the South Kavirondo District of Nyanza Province that there was correlation between fly-density (*S. neavei*) and the incidence of onchocerciasis in the people; and since the range of flight and occurrence of the fly were limited by ecological factors, a "pocketing" of the disease could thus be inferred which would be a favourable factor in any control scheme. The obvious remedy of evacuating a population from an infected locality to an area where the insect vector was unknown, did not recommend itself, for various reasons. Moreover, the subsequent re-introduction of a "clean" population into an evacuated area in which the infection in the flies had died out would necessarily presume for its success on the absence of any animal reservoir hosts which might have continued to infect a new generation of "clean" flies in the absence of the human hosts; and such a presumption had no justification. That the possibility of control existed, however, was recently borne out by the dramatic and highly successful results achieved by Garnham and McMahon (1947) in eradicating *S. neavei* from the afflicted area referred to above, by means of an anti-larval measure consisting of treatment of water courses in the infected "pocket" with D.D.T. emulsion. The importance of this work requires no emphasis since it holds out a prospect of eradicating onchocerciasis in all areas where *S. neavei* is the vector. But it also holds a scientific significance of great interest, for it throws new light on the perplexing problem of the breeding habit of *S. neavei*. It not only confirms the belief that *S. neavei*, like other Simuliidae, breeds in running water, but it also confines the possible range of breeding to those waters flowing through the area inhabited by the adult flies and downstream from these areas. No explanation, however, is yet forthcoming to account for the fact that the larvae and pupae of *S. neavei* have not yet been found in spite of repeated investigations in waters where they are now known, as a result of Garnham's and McMahon's experiment, to have been breeding. From the practical standpoint this hiatus has diminished greatly in importance since it is now possible to destroy the unseen and as yet unknown larval stages. Academically, it remains a problem of absorbing interest whose solution should provide information of much entomological significance.

The present enquiry was undertaken in 1941 at the instance of the Colonial Office by arrangement between the Government of Kenya and the London School of Hygiene and Tropical Medicine. The writer wishes to acknowledge his indebtedness for the co-operation of the

Medical Department and Administration of the Colony throughout the period of enquiry and to express his gratitude to Dr. A. R. Paterson, then Director of Medical Services ; to Dr. P. C. C. Garnham, then in charge of the Division of Insect-borne Diseases, and to Mr. P. J. McMahon and Mr. W. Grainger, also of this Division, for their generous assistance at all times.

Results of Preliminary and Mass Onchocerciasis Surveys in Native Populations of Nyanza Province.

Description of the Area.

Nyanza Province occupies a sector of the western part of Kenya Colony where Lake Victoria intrudes into it as the Kavirondo Gulf. It is bisected by the equator, to the north and south of which it extends for about 70 miles. From its lowest altitude at lake level it rises to the east where a mountain range attaining over 9,000′ separates it from the rest of the Colony and to the north it is bounded by the high country which ascends at Mt. Elgon to 14,140′. This configuration results in a system of rivers arising in the east and north-eastern elevations and flowing into the Gulf and the Lake. (See Map II.) The system comprises four principal rivers, of which the largest and most northerly, the Nzoya River, has its catchment both in the Mt. Elgon massif and in the easterly range. The others, the Yala, Sondu and Kuja rivers respectively, arise only in the east.

It will be seen from the simplified contours on the map, that the transition from high country to lake level is not uniform, but that in general there is a steep fall from 8,000′ to 5,000′. Below this level the terrain tends to flatten out, resulting in very gradual sloping and in most places wide expanses of plainland. The effect of this on the river systems is to vary their character at different altitudes. Whereas in the upper reaches they are fast-flowing, richly served with rapid tributaries and waterfalls are frequent, in the lower reaches these characteristics are reversed.

Afforested areas occur mainly above 6,000′ and are represented by the Chepalungu forest and Mau forest to the south and east respectively ; but the greater part of the Kakamega-Kapwaren tract lies between 5,000′ and 6,000′. Elsewhere at this altitude forest or thickly wooded areas are much reduced and only appear as isolated patches or narrow discontinuous stretches along the banks of certain rivers. Scattered bush and scrub characterise the vegetation of the lowland plains, where even thinly-wooded river banks are rarely seen.

The native population within the Province comprises several tribes

of diverse origin. The Luo, of Nilotic stock, predominate numerically and inhabit the southern, central and northern regions, or Kavirondo Districts. In Lumbwa District to the south-east are the Kipsigis, a virile off-shoot of the Nandi, who migrated from the rocky northern regions in early days and ousted the Masai and Kisii from the fertile Lumbwa country, wherein they settled as agriculturists and thus earned the derogatory title of " Lumbwa " from the Masai, who are essentially a pastoral tribe. In South Kavirondo the Kisii tribe (Bantu) inhabit an area defined by Luo country to the north and west and by their traditional enemies the Kipsigis in Lumbwa District to the east. Central Kavirondo, situated to the east and north of the Gulf, is inhabited mainly by Luo and to a lesser extent by Teriki and Nandi tribes.

Preliminary Survey in South Kavirondo District and Lumbwa District.

Population samples, numbering on an average 137, were examined for onchocerciasis in eighteen administrative Locations in the South Kavirondo District, and in four localities in Lumbwa District. The results of the survey are depicted in Map I, which shows the Locations or localities in which the people were assembled and examined and the percentage infection found in them. The examinations comprised people of the Luo, Kisii and Kipsigis tribes and were conducted *in loco* at altitudes varying from below 4,000′ to nearly 7,000′ and thus included represented kinds of terrain in the Districts, from the fertile but thinly-wooded plains approaching lake level to the thickly-wooded highlands of Lumbwa.

The survey revealed some interesting facts concerning the distribution of the infection. Outstanding was its presence at the intermediate levels ranging from just below 5,000′ to just below 6,000′, and its absence in the low-lying plains and at the higher altitudes. Before discussing the factors affecting this distribution the details of the survey will be described.

Method of Examining Skin for Microfilariae. In this preliminary rapid survey the data obtained were based on the evidence of cutaneous onchocerciasis as manifested by the presence of microfilariae of *O. volvulus* in small slices of skin removed from the forearm. A simple procedure was adopted which obviated the need for local anaesthesia and the use of complicated and bulky equipment such as centrifuges and glass containers. The method of removing the skin slice, which was devised and described by the writer (1939) for diagnosis of

O. gibsoni in cattle in Malaya, consisted of pinching up the skin with thumb and forefinger of one hand and slicing off, with a sharp razor blade, a *very thin* piece of skin about 5 mm. in diameter. In mass examinations in the field the most rapid and convenient procedure was to take the skin slice from the forearm and immerse it in a drop of normal saline on a numbered 3″ by 1″ slide. This was examined under a magnification of x230 a few minutes later, when microfilariae if present in the skin would have emerged and were easily detected swimming in the saline. Usually 5 or 10 such slides were prepared at a time, thus allowing a suitable interval between immersion and examination.

Distribution of Microfilariae in the Skin. The forearm was chosen as a matter of convenience. A preliminary attempt had been made to determine the most favourable part of the body to examine, i.e., which would give the largest yield of microfilariae, with rather anomalous results. An adult male Luo, having what appeared to be a heavy skin infestation with microfilariae, was selected for this trial. Skin-snippings of more or less uniform size and thickness were taken from various parts of the body and the number of microfilariae emerging from each was counted, with the following result. Duplicate skin-snips were taken, about 3 cms. apart, from each part of the body.

Location of skin-snip.	*Number of Microfilariae.*	*Average number per skin-snip.*
Neck (right side)	67 and 81	86
,, (left ,,)	150 ,, 45	
Right forearm	31 ,, 46	181
Left ,, ...	369 ,, 277	
Right thigh	3 ,, 11	102
Left ,, ...	393 ,, 2	
Right leg	71 ,, 51	45
Left ,, ...	36 ,, 18	
Back (4″ to right of backbone)	106 ,, 42	48
,, (4″ to left of backbone)	42 ,, 2	

The discrepancies in these findings indicated that the skin-snip method as described could not be relied upon for a quantitative estimate of the degree of skin infestation.

Population Samples Examined in S. Kavirondo District.

In South Kavirondo District, the administrative Locations in which population samples were examined are indicated in Map I together with the incidences of infection, which are expressed as percentages

(to the nearest integer) inset in light or heavy circles. The heavy circles (relatively high incidence) represent samples of *endemic* onchocerciasis; subsequent data showed that the lower figures of 1% to 7% in light circles did not represent a low-grade endemicity but included positive cases who must have acquired the infection in one or other of the endemic foci.

The numbers of people in each sample and the numbers positive were as follows. The sequence in which the Locations are taken roughly follows a geographical trend from the lower to the higher altitudes.

A. *Kaniamwaa* (*Luo*). Examined 131 (95 males, 36 females; 112 adults, 19 children). All negative.

B. *Kanyada* (*Luo*). Examined 112 (78 males, 34 females; 102 adults, 10 children). All negative.

C. *Kochia* (*Luo*). Examined 152 (74 males, 78 females; 125 adults, 27 children). Ten positive (6 males, 4 females)—6.6%.

D. *Karachonyo* (*Luo*). Examined 150 (57 males, 93 females; 115 adults, 35 children). All negative.

E. *Kabwoch* (*Luo*). Examined 190 (113 males, 77 females; 149 adults, 41 children). All negative.

F. *Gem* (*Luo*). Examined 150 (97 males, 53 females; 132 adults, 18 children). Nine positive (6 males, 3 females)—6%.

*G. *Mumbo* (*Luo*). Examined 117 (94 males, 23 females; 83 adults, 34 children). Thirty-three positive (27 males, 6 females)—28.2%.

H. *Kabondo* (*Luo*). Examined 151 (103 males, 48 females; 128 adults, 23 children). All negative.

*I. *Mukseru* (*Kisii*). Examined 120 (91 males, 29 females; 57 adults, 63 children). Twenty-five positive (18 males, 7 females)—20.8%.

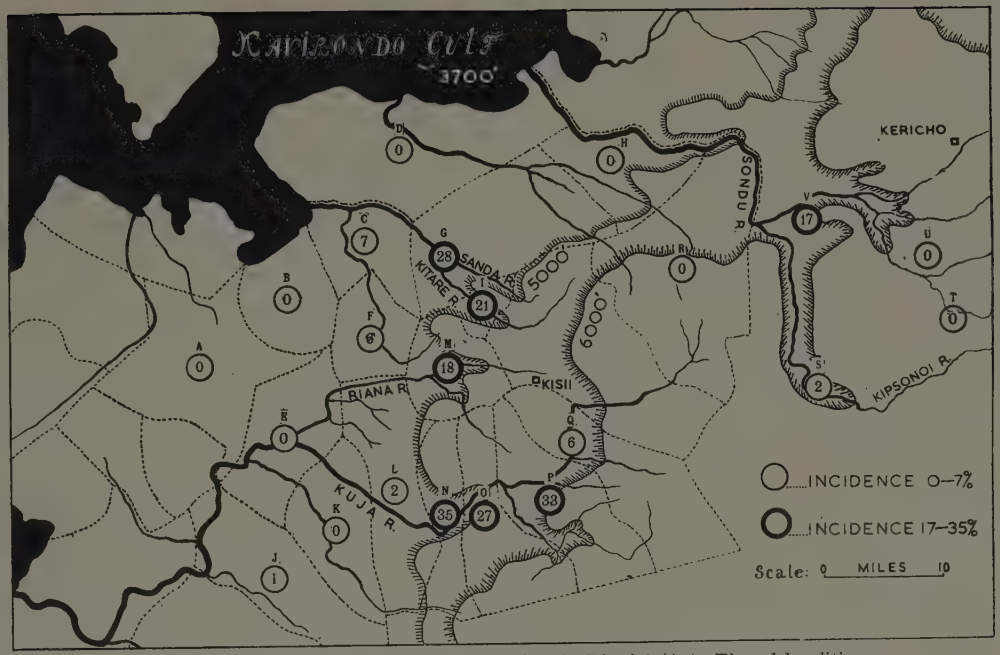
J. *Kaniamkago* (*Luo*). Examined 174 (152 males, 22 females; 162 adults, 12 children). One positive (1 male)—0.6%.

K. *Sakwa* (*Luo*). Examined 120 (53 males, 67 females; 109 adults, 11 children). All negative.

L. *Kamagambo* (*Luo*). Examined 95 (50 males, 45 females; 90 adults, 5 children). Two positive (1 male, 1 female)—2.1%.

M. *Wanjare* (*Kisii*). Examined 234 (174 males, 60 females; 145 adults, 89 children). Forty-one positive (24 males, 17 females)—17.5%.

*Mumbo and Mukseru are now merged into one location—Casipol.



Map I. Showing Locations in South Kavirondo District (A to R) and localities in Lumbwa District (S to V) in which population samples were examined for onchocerciasis. Figures in circles are the percentages found infected (to the nearest integer). Lettering as on pages 6, 8 and 10.

N. *South Mugirango* (*Kisii*). Examined 118 (94 males, 24 females ; 102 adults, 16 children). Forty-one positive (29 males, 12 females)—34.7%.

O. *Majaoge* (*Kisii*). Examined 149 (97 males, 52 females ; 117 adults, 32 children). Forty positive (28 males, 17 females)—26.8%.

P. *Bassi* (*Kisii*). Examined 141 (126 males, 15 females ; 114 adults, 27 children). Forty-six positive (40 males, 6 females)—32.6%.

Q. *Nyeribari* (*Kisii*). Examined 103 (96 males, 7 females ; 79 adults, 24 children). Six positive (6 males)—5.8%.

R. *North Mugirango* (*Kisii*). Examined 63 (56 males, 7 females ; 56 adults, 7 children). All negative.

From this preliminary survey it was possible to define three distinct foci of the infection, each having certain topographical characteristics in common which determined not only the existence of the disease but also its geographical limits. The West Mumbo and Mukseru Locations samples were from the endemic area (Kodera) previously reported on by McMahon (1940). The Wanjare Location sample represented a smaller focus (Riana) which was hitherto unrecorded. The samples from Locations South Mugirango, Majaoge and Bassi represented a larger but less compact focus (Kuja River focus) which had been suspected by the local authorities and had been the subject of a preliminary investigation by McMahon, who had personally communicated his findings to the writer. Concurrently with this infection incidence survey, all the rivers in each Location were searched for *Simulium*. Biting adults were sought for, using human bait on river banks, and the waters were searched for evidence of breeding forms, by collecting pupae on rocks and on vegetation. *Simulium neavei* had already been indicated by McMahon (1940) as the vector in the Kodera focus, and it was significant, in this present preliminary survey, that this was the principal *Simulium* species found biting humans. Moreover, it was found in all those Locations having a relatively high incidence with onchocerciasis ; elsewhere it was absent or extremely scarce, probably merely a sporadic occurrence. Pupal findings comprised a good variety of species which will be listed later. None of these included the pupae of *S. neavei*, yet somewhat paradoxically included that of *S. damnosum*, which was surprisingly rare or absent as a human biter even on rivers in which its pupae were very commonly found.

Three fundamental ecological requirements of *S. neavei* were immediately discernible during the survey in S. Kavirondo District. 1. The presence of fast-running rivers or streams. 2. A hilly or mountainous terrain. 3. Well-wooded river banks. Locations in which one or more of these topographical features were lacking, produced no *S. neavei* and the onchocerciasis incidence was accordingly nil or very low. Thus, in Locations A, B, C, D, E, F, H, J, K and L having a very low or negative incidence of the disease and a complete absence of *S. neavei*, the terrain is level, the rivers are accordingly slower and lacking in waterfalls or rapids, and the river banks are either devoid of trees or are thinly-wooded. In Locations G, I, M, N, O and P, with a " high " incidence and inhabited by *S. neavei*, the three ecological factors are concurrent. In Locations Q and R, without *S. neavei* and with a low or negative incidence of onchocerciasis, factors 1 and 2 are present but the third factor is notably absent. The appreciation of the significance of these factors in relation to the distribution of onchocerciasis and its vector rendered unnecessary the continuance of the survey in the other Locations for which no data are included in Map I.

Proceeding with the survey from S. Kavirondo further east into Lumbwa District, the endemic area discovered by Dry in 1921 was quickly located (V. Ngoina, Map I) and a population sample of 176 (127 males, 49 females ; 151 adults, 25 children), examined by the skin-snip technique, revealed an onchocerciasis incidence of 16.5%. This area is situated at an altitude slightly under 6,000′ and embraces portions of two large rivers, the Chemangat (or Yurith) and the Kipsonoi, and their tributaries. From their confluence, forming the Sondu River (or Miriu), the area extends eastward for about 10 miles with a depth of about 3–4 miles. It is well wooded with a good variety of trees, of which species of thorn are a characteristic feature. The most striking feature, however, was the occurrence in certain densely-wooded small tributaries of enormous numbers of *S. neavei*, compared with which the incidence observed in the endemic localities of S. Kavirondo paled almost into insignificance.

From this area the survey was continued further east into the high country approaching 7,000′, which is also well wooded and widely traversed by several rapid streams which form the tributaries and watershed of the Chemangat and Kipsonoi Rivers. At Kimulot, on the Kiptiget River (U. Map I), 74 people were examined. All were negative. At Mogogosiet, near the Itare River (T. Map I), 79 were examined and these were also negative. These findings were in keeping

with the results of searches for *S. neavei*, of which only one or two specimens were caught after hours of patient waiting.

The absence, or extreme scarcity of *S. neavei* at these higher altitudes where the three factors previously postulated for its occurrence are notably in evidence, implies that a fourth factor is concerned in the ecology of *S. neavei*. It might be surmised that the inhibiting factor, or at least one of them, is the lower temperature which occurs at the higher altitudes. It is, indeed, very probable that this is so, for in every other respect the locality appears ideal as a *S. neavei* habitat, with its fast-running streams and densely-wooded banks in a hilly terrain which fulfilled all the conditions observed previously wherever *S. neavei* had been found. But until more is known about the breeding habits of this species the true explanation must remain in doubt.

At Chemagel, at a slightly lower altitude (6,000′) on the Kipsonoi River (S. Map I), a population sample of 85 was examined, which gave 2 positives. Prolonged search on a lightly-wooded stretch of the Kipsonoi River in the locality—the only one of its kind—showed that *S. neavei* was present, but in extremely small numbers.

Population Samples Examined in Lumbwa District.

S. *Chemagel* (*Kipsigis*). Examined 85 (63 males, 22 females; 71 adults, 14 children). Two positives (adult males)—2.4%.

T. *Mogogosiet* (*Kipsigis*). Examined 79 (70 males, 9 females; 77 adults, 2 children). All negative.

U. *Kimulot* (*Kipsigis*). Examined 74 (67 males, 7 females; 56 adults, 18 children). All negative.

V. *Ngoina* (*Kipsigis*). Examined 176 (127 males, 49 females; 151 adults, 25 children). Twenty-nine positives (21 males, 8 females)—16.5%.

Mass Survey in Four Endemic Localities.

In the preliminary survey, described above, which was designed to discover the geographical distribution and ecology of the infection, the numbers of people examined were necessarily small; and although the survey served its purpose in these respects, it was apparent that the incidences recorded in the endemic localities did not afford a true index of the population infection. Accordingly, mass examinations were undertaken in four endemic localities, namely Riana, Kodera, Lumbwa and also in the Kakamega-Kapwaren forest region in North Kavirondo which had been studied by Hawking (1940). It was hoped in this survey to obtain further information concerning age and sex incidence,

and to make observations on the relation between incidences and clinical effects, in so far as was possible, although medical skill in the clinical aspects of onchocerciasis was unfortunately not easily available. In particular, difficulty in the accurate diagnosis of onchocercal blindness or eye disease as distinct from other diseased eye conditions was anticipated. The skin disease associated with onchocercal infection also was not always clearly distinguishable from other abnormal skin conditions, especially in older people. Onchocerca nodules were more easily assessable but required rather prolonged and detailed examinations which could not invariably be carried out.

The Kodera Focus.

This endemic area embraces sections of the Kitare and Sanda Rivers over a distance of about 7 and 6 miles respectively and extends from their confluence in a south-easterly direction to where they are crossed by the Kisii-Oyugis Road. People from three sub-Locations were examined, two of which, Kaniago and Anyiko, contain the endemic section of the Kitare River and one of which, Kunuongo, contains part of the Sanda River section. Incidence data from the three sub-Locations are presented separately.

Skin positives. Of 705 people examined in sub-Location Kaniago, 52.6% were shown positive by the skin-snip method. The percentage in males was 57.1% and in females 42.3%. The percentage in different age groups is illustrated in Graph I, which shows a progressive rise in the infection rate with age.

Of 491 examined in sub-Location Anyiko, 42.3% were positive and again the percentage in males (47.7%) exceeded that in females (36.2%). The age-group curve shows a steep upward trend similar to that of Kaniago.

Of 336 examined in sub-Location Kunuongo, 16.6% were positive, males showing 21.2% and females 9.3%. These people inhabit an area which includes the lower part of the Sanda River but extends a considerable distance away from the river, which explains the comparatively low infection rate.

Ocular Complications. Defective eyes or eyesight was noted in 77 of 1,196 people from Kaniago and Anyiko. Information was received from the headman concerning 69 others who were said to be unable to come to be examined as they were confined to their huts by blindness. A probable total of 146 is assumed from this. Detailed examinations of the eyes of 44 of the 77 were carried out by Dr. H. Murcott who considered that in about 35, or 80%, of the cases, the condition was

due to onchocerciasis. It may be inferred from these figures that in at least 10% of the people ocular complications of onchocerciasis are present.

The cases diagnosed by Dr. Murcott ranged from 11 to 60 years in age ; about $\frac{2}{3}$ of them were aged 30–40 years and the number of males was twice that of females. The principal eye lesions observed were keratitis (17 cases), iridocyclitis (5 cases), and oval pupil (5 cases). Iritis, conjunctivitis and photophobia were also present in several. Skin snips were positive for microfilariae in nearly all those showing onchocercal eye lesions but nodules were only seen in seven.

Of the 336 people from Kunuongo sub-Location (Sanda River) 27 had defective eyes or eyesight but only 10 of them showed positive skin-snips. Assuming on the basis of the Kaniago and Anyiko findings that 80% (or 22) of these were onchocercal in origin, the figure of 6.5% is arrived at as the percentage of Kunuongo people having onchocercal eye complications. This relatively small proportion of eye-affected people having positive skin snips suggests that *less* than 80% of the 27 were onchocercal and hence that 6.5% may be an over-estimate for Kunuongo.

TABLE I.

Summary of Microscopical and Clinical Data from Kodera.

Locality	*Number examined*	*Mf. + in skin-snip*	*Ocular complications*	*Onchocerca nodules*	*Abnormal skin*
Kaniago and Anyiko	1,196	48.4% M. 54.9% F. 39.6%	9.8%	4.7%	15%
Kunuongo	336	16.6% M. 21.2% F. 9.3%	6.5%	3.2%	14.6%

Onchocerca Nodules. In Kaniago and Anyiko, onchocerca nodules were observed in 56 (or 4.7%) of the total 1,196 examined. (Microfilariae were present in the skin of 47 of the 56 cases). This percentage is considerably lower than that observed by Harris (1940) in 406 unselected natives from the Kodera area, and is attributable to the less exhaustive examination for nodules which was carried out in the present survey.

In Kunuongo, nodules were observed in 11 or 3.2% of the 336 examined. Microfilariae were present in the skin of 7 of the 11 cases.

Abnormal Skin. In Kaniago and Anyiko 177 or 15% of the people who were skin-snipped showed abnormal skin. 130 or 73% of these had microfilariae in their skin.

In Kunuongo people, the number with abnormal skin was 49 in 336, which gives a percentage, 14.6%, similar to the previous one but only 18 or 36.7% of the 49 showed microfilariae in their skin.

The difficulty of diagnosis of skin abnormalities due to onchocerciasis has been referred to and it might be inferred from these figures that by no means all of such cases were due to this cause, since in Kunuongo people, having a much lower incidence of onchocerciasis than Kaniago or Anyiko, there was a similar percentage of abnormal skins and no correlation between abnormal skin and the presence of microfilariae.

The Riana Focus.

This very small focus is situated in Wanjare Location about 6 miles south of the " Kodera " area and is inhabited by natives of the Kisii tribe. The infected area, *i.e.*, the distribution range of adult *Simulium neavei*, comprises a densely wooded 2-mile stretch of the Riana River and a similar 1½ miles of the Yabe River whose confluence with the Riana River marks the westerly boundary of the infected area. These densely wooded stretches terminate abruptly in the confluence of the two streams and upstream from there for the distances mentioned. *Simulium neavei* occurs only in these limited stretches and is present in comparatively small numbers. Its incidence was observed there over a long period and will be discussed subsequently.

With regard to the disease, the natives inhabiting this area, unlike those of the Kodera area, do not complain much about it in any of its manifestations, and the mass examinations carried out revealed a relatively small number of positive skin snips and few cases of eye-disease. The results are summarised in Table II. It is of interest to note that the incidence in males is only very slightly greater than in females in this population.

Table II.

Summary of Microscopical and Clinical Data from Riana.

Number examined	*Mf. + in skin-snip*	*Ocular complications*	*Onchocerca nodules*	*Abnormal skin*
798	21.2%	1.6%	3.3%	15.2%
	M. 22.4% F. 20.6%			

The Lumbwa Focus.

This infected area is less clearly circumscribed than either the Kodera or Riana focus, inasmuch as the transition from *S. neavei*-infested to non-infested terrain is gradual. There is, however, a sudden drop in the incidence of *S. neavei* outside the boundaries defined on page 9 and the mass examination was confined to those people living near to or within easy range of rivers having a relatively high *S. neavei* incidence. The result of the examination is summarised in Table III.

The incidence of the infection in this focus (28.8%)is considerably higher than at Riana but very much lower than in the Kodera area. Ocular complications are also on a much lower scale, as is evident from the low figure of 1.8% in the survey as well as from general observations on the people during a prolonged stay in this focus. Although the disease, as pointed out by Dry (1920) in both skin and eye manifestations is well known to the people it has never assumed the serious proportions for which it is notorious in the Kodera area where it gave rise to the epithet of the " country of the blind."

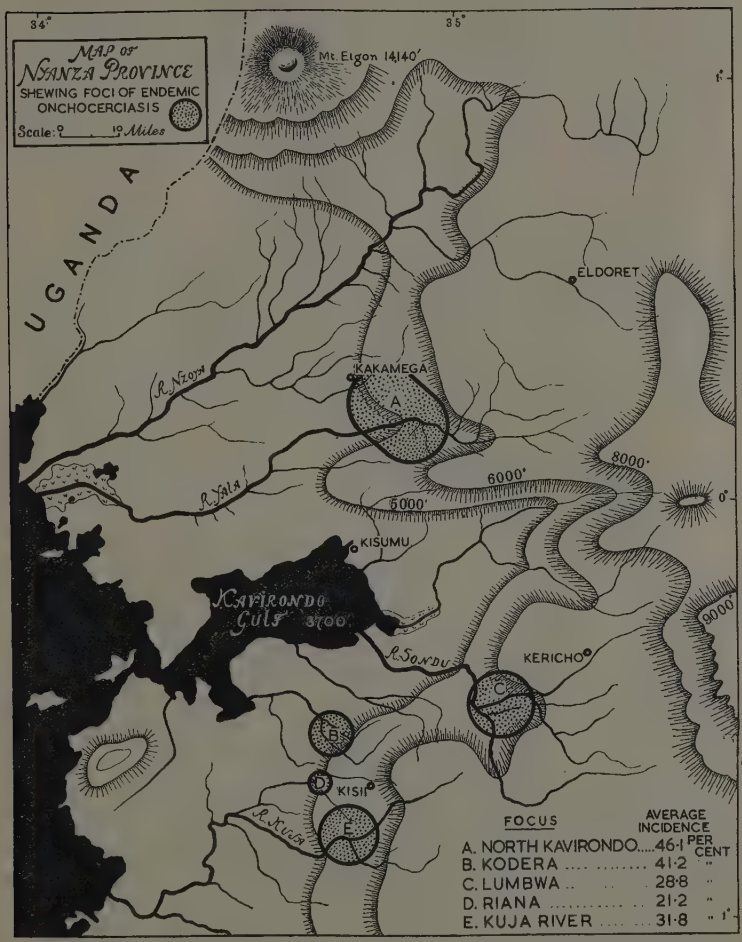
Table III.

Summary of Microscopical and Clinical Data from Lumbwa.

Number examined	*Mf. + in skin-snip*	*Ocular complications*	*Onchocerca nodules*	*Abnormal skin*
834	28.8% M. 35.2% F. 21%	1.8%	3.3%	8.8%

The North Kavirondo Focus.

This is the largest of the five endemic foci discussed in the present report. It is centred mainly about the Yala River and its tributaries where they flow between the 6,000′ and 5,000′ contour lines and it is at this level that the river flows through the extensive tract of the Kakamega-Kapwaren forest which extends north and south of it to a depth of about 20 miles. While the boundaries of the focus are roughly definable to the east, by the abrupt ascent at 6,000′ ; to the south by the cessation of suitable forest and woodland ; to the west by the transition from hilly terrain to plainland, the limiting factors are not clear to the north where tributaries of the Nzoya River system as well as those of the Yala River are embraced by the Kakamega forest and some of these are also infested with *S. neavei*.



Map II. Illustrating semi-diagrammatically some of the principal topographical features of Nyanza Province and their relation to the five endemic foci of onchocerciasis.

A complete population survey in this large area being impracticable, population samples were examined from three localities, one of which, Kaimosi, was situated near the centre of the focus and two, Mohotu and Chaburinga, close to its western periphery. The results of these examinations are summarised in Table IV. The Mohotu and Chaburinga results are combined in the table as separately they are substantially the same.

Kaimosi.

Skin positives. The unselected population sample shows some exceptional features with regard to the infection incidence. Not only does the average incidence (71.8%) far exceed the previous highest figure of 52.6% at Kaniago in S. Kavirondo, but the incidence in the younger age groups is about as high as in the older. Thus of 50 aged 1–19, 70% were positive ; of 58 aged 20–39, 70.7% were positive and of 34 aged over 40, 76.5% were positive. The close approximation of the incidence in sexes is also notable.

TABLE IV.

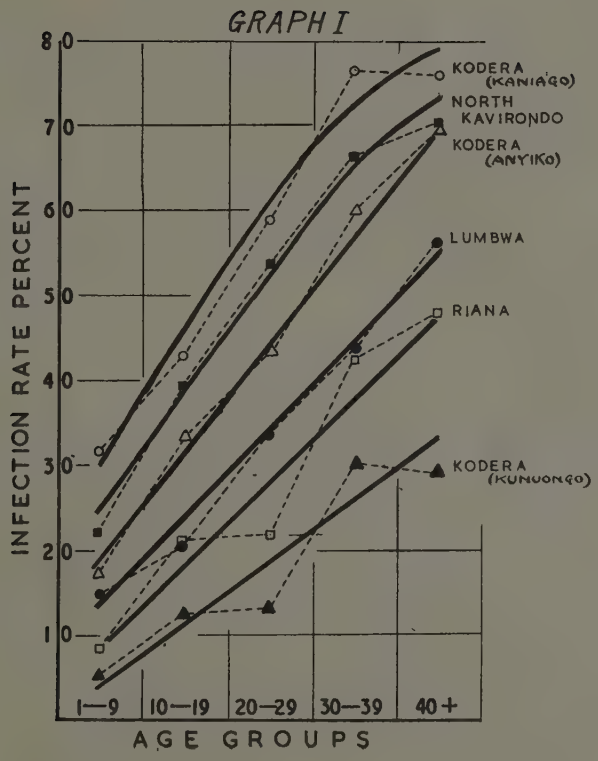
Summary of Microscopical and Clinical Data from North Kavirondo.

Locality	*Number examined*	*Mf. + in skin-snip*	*Ocular complications*	*Onchocerca nodules*	*Abnormal skin*
Kaimosi	142	71.8% M. 72.8% F. 69.2%	10.5%	24.4%	25.1%
Mohotu and Chaburinga	331	35% M. 41.3% F. 27.2%	2.7%	8.5%	9.97%

Ocular complications. It is probable that the figure of 10.5% is an under-estimate since it was possible to assemble, without much difficulty, ten additional cases from this locality. These were examined by Dr. P. C. C. Garnham who observed indications of blindness of onchocercal origin in six.

Onchocerca nodules. The comparatively high incidence of people with nodules is also notable and may be correlated with the high incidence of skin positives. The situation of the nodules was on the ribs, hips and knees in equal proportions of cases.

Abnormal skin. The percentage of people with abnormal skin, like the other conditions, is high in comparison with other localities and the


GRAPH I
INFECTION RATE PERCENT
80
70
60
50
40
30
20
10
KODERA (KANIAGO)
NORTH KAVIRONDO
KODERA (ANYIKO)
LUMBWA
RIANA
KODERA (KUNUONGO)
1—9
10—19
20—29
30—39
40+
AGE GROUPS

occurrence of microfilariae in the skin of 33 out of the 36 cases implies that these skin abnormalities are of onchocercal origin.

Mohotu and Chaburinga.

The results of examinations in these two localities do not call for special comment except that the infection rate in age groups, unlike Kaimosi, follows the usual upward curve and there is a sharp difference in the sex incidence. (It is of interest to note that the average infection rate in these two outlying localities (35%) is close to that found by Hawking (1939) in 53 unselected natives examined by the skin-snip method, 38% of whom were positive.)

A comparison of the present figures with those of Kaimosi (Table IV) suggests that a high infection rate by skin-snip examination is accompanied by a high proportion of cases having the other manifestations of onchocerciasis and that a low proportion of such cases accompanies a low infection rate.

Examination of Gold-mine Labourers for Onchocerciasis.

McAlder Mine.

This large and important mine is situated in Kadem Location in S. Kavirondo, on the lower reaches of the Kuja River about 10 miles inland from the Lake. 241 adult males of the labour force were skin-snipped and only two were found positive. One of these came from Tanganyika and the other from Sakwa Location. Most of those examined had been recruited either from Kadem itself or from neighbouring Locations adjacent to the Lake, such as Mohoru, Karungu, Kwabwai, Gwassi, Kaksingiri, Kasigunga, Kanyada, Karachwonyo, Nyakatch, Kaniamwaa, Kenyadoto, etc., and their freedom from infection merely confirmed the conclusions derived from the preliminary survey in S. Kavirondo, namely that, *O. volvulus* is not transmitted at the lower altitudes in this District.

Stitt's Mine.

This is a small alluvial mine situated on the Yala River in N. Kavirondo about 10 miles west of the main road from Kakamega to Kisumu. 39 of the adult male workers were examined and 8 were positive. The true origin of their infections was doubtful since these men had worked elsewhere from time to time. The examination at this spot of 37 children, all of whom were negative, indicated that this part of the Yala River is outside the endemic focus which embraces the

river to the east. Searches for *S. neavei* in the vicinity of the mine proved negative.

Distribution of gold mines in relation to endemic foci. With the increasing knowledge furnished by the surveys of Hawking (1939) and McMahon (1940) as to the geographical distribution of the infection in Nyanza Province, some concern was expressed that gold-mining activities might be adversely affected directly or indirectly. The better understanding of the nature and ecology of the infection was reassuring with regard to its possible spread to localities hitherto immune, but the existing distribution of mines in some instances appeared to overlap or approximate to that of the infection. The position in 1944 was that there were about 40 mines, of which less than half were at that time working. Most of these are situated in Central and North Kavirondo. In South Kavirondo, the small group on the lower Kuja River (which includes McAlder Mine) is far removed from any endemic focus. Higher up this river is Kitere Mine which is just outside the Kuja River focus. At Oyugis, a few miles to the north east of the Kodera focus, there are two small mines which are unaffected, but prospecting in this promising locality was hindered before the advent of D.D.T. control made it inocuous. In Central and North Kavirondo most of the mines lie to the west of the Kakamega-Kisumu road and may be regarded as outside the range of infection. A group of about 9 mines lying to the east of the road, within or close to the infection zone, appear to be exposed to the risk of visits by infected *S. neavei*, which also might be sporadic visitors further west.

The Relation of Age and Sex to Infection by *O. volvulus*.

Age incidence. During the surveys described above, the age of each person examined was estimated and noted. Although such estimations are necessarily approximate only, they proved to be accurate enough, when classified in age-groups of ten years each, to reveal an interesting trend of the infection incidence in the population. It is clear from the examples shown in Graph I that there is a progressive increase in the proportion of infected to non-infected persons, from the younger age-groups to the older. In other words, in an infected population, one is more likely to find microfilariae in the skin of an old person than in a young one. The explanation of this probably involves at least two factors, first, that older persons have been exposed to infection for a longer time and hence are more likely to be infected than are younger persons ; and secondly, they are more likely to have been infected more

frequently and to have larger numbers of microfilariae in their skin. This gives greater accuracy to the skin-snip technique, and hence, a smaller proportion of false negatives due to scanty microfilariae, will be recorded. In the younger age-groups the converse of all this will hold.

It is of interest to note that this age-to-incidence ratio also occurs within the youngest age-groups. Thus of 344 children aged 2 to 10 years from Kaniago and Anyiko, those aged 2–4 years showed 18.9% positive in 116 examined ; those of 5–7 years showed 28.7% in 115 ; and those of 8–10 years showed 34.5% in 113. Children under two years of age were not examined. Only one child aged 2 years was positive, indicating that in a heavily infected population microfilariae first appear in the skin in appreciable numbers of children at the age of 2 to 3 years. (The beneficial effects of the clearance of *S. neavei* from the Kodera area (Garnham and McMahon, 1947) should on this reckoning become observable in the young children of that area in a few years' time.)

Sex incidence. In each of the four foci surveyed, the average incidence in men exceeds that in women. This difference is statistically significant in Kodera, Lumbwa and North Kavirondo where it may be ascribed to occupation, since the women's domestic duties tend to confine them more to their huts, which are never invaded by *S. neavei*, whilst the outdoor habit of the males exposes them more to the attacks of *S. neavei* in the rivers and in the woods. This explanation fails however to account for the sex distribution of the infection in the Riana population, in which the difference between the sex incidence is *not* significant ; in fact, in the older age-groups the females show a higher incidence than the males. The relatively high incidence in females seems to be a characteristic of the Kisii tribe, for in the preliminary survey in Mukseru, Majaoge, Bassi and South Mugirango, all of which are inhabited by Kisii, the figures suggest a predominence of the infection in females. McMahon (1940) also noted and commented upon this difference. But there is no obvious difference in tribal custom or habit, or in the environmental conditions of the country inhabited by the Kisii, to which this difference in sex-incidence might be ascribed. An even more puzzling feature of the Riana figures is the statistically significant fall of the infection incidence in the 20–29 years age-group in males (Graph III). A similar fall is seen in the N. Kavirondo graph, but in this instance the uneven distribution does not differ significantly from a chance distribution. In each of the four graphs it will be noted that more male children in the 1–9 group are infected than are females. This results from occupation, for small boys at an early age go out in the field to ten cattle and goats and are thus more exposed to infection.

GRAPH II
KODERA
GRAPH III
RIANA
GRAPH IV
LUMBWA
GRAPH V
NORTH KAVIRONDO
INFECTION RATE PERCENT
80
70
60
50
40
30
20
10
♂
♀
1—9 10-19 20-29 30-39 40+
AGE GROUPS

Discussion.

The foregoing account of a survey of onchocerciasis in Nyanza Province confirms the importance and magnitude of the infection already indicated by previous surveys, but reveals, in addition, a comparative unevenness in the degree of importance in different foci. This is reflected especially in the results of the uniform application of a standardized technique of skin examination for the presence of microfilariae, which show up remarkable differences in the average infection rate of populations from different foci. Thus in the Kodera focus the incidence was found to be 41.2% whilst at Riana, less than 10 miles distant, it is only 21.2%. A comparison between the two foci, in respect to the various factors relevant to the man-insect contact, such as occupation, habit, custom, terrain, topography, reveals no obvious single explanation for this difference, which therefore may be due to a combination of circumstances as yet not fully understood; or it may be due to differences in fly infectivity, a possibility which will be discussed in a subsequent paper. The simplest explanation, that of fly-density, fails to account for it. Extended observations on the fly-incidence in the two places during the period of the present survey indicated that *S. neavei* was on an average no more abundant at Kodera than at Riana. Moreover, at the Lumbwa focus, where the infection incidence, 28.8%, is intermediate between that of Riana and Kodera, the fly density is exceedingly greater than in either of these places. On the other hand, McMahon (1940) found that there was locally a correlation between fly-density and human infection incidence **in different places *within* the Kodera focus.**

Concerning the more serious effects of the infection, there is also considerable variation in importance from focus to focus. Thus, in Kodera and North Kavirondo, eye complications are much more in evidence than in the Lumbwa and Riana foci. Correlated with this, is the incidence of the infection in these populations, for in the former two foci it is over 40% while in the latter two it is less than 30%. It is possible to account for this correlation by invoking two conditions, (*a*) that eye-disease is more likely to occur in a heavily-infected person, *i.e.*, one who has been repeatedly bitten by infected flies and (*b*) that in a community having a high average infection rate the opportunities for re-infection are proportionately more frequent.

Finally, the age-group analysis, which displays very clearly the cumulative effect of exposure to infection over a long period (and incidentally argues the absence of premunity in onchocerciasis) suggests

that it is necessary to live a long time in a heavily infected area in order to run the risk of incurring the more serious forms of the disease; conversely, that during a short sojourn of a few years, unless there is abnormal exposure to the bites of *S. neavei*, dangerous consequences are unlikely to ensue.

Summary.

1. A survey of the incidence and distribution of *Onchocerca volvulus* in Nyanza Province was carried out by the " skin-snip " method of determining the presence of microfilariae in the skin. 5,842 Africans were examined.

2. An account is given of the incidence of the infection in five different foci, with some observations on ocular complications, cutaneous nodules, abnormal skin, and age and sex incidence.

3. The survey disclosed one hitherto unrecorded focus of the infection, namely, at Riana in South Kavirondo District and confirmed the existence of another on parts of the Kuja River in the same district. The endemic focus discovered in Lumbwa District by Dry in 1921 was surveyed for the first time.

4. Each focus is fairly well circumscribed and all are situated at an altitude roughly defined by the 5,000′ and 6,000′ contour lines. Both of these limitations are determined by topography and by the insect vector, *Simulium neavei*, whose habits confine it to a certain type of environment. This is characterized by the presence of fast-running rivers or streams; a hilly or mountainous terrain; well-wooded river banks.

5. The incidence of the infection in people increases progressively with age and in a heavily infected community (*e.g.* Kaniago) ranges from about 80% in the 1–9 age-group to over 75% in the 40+ age-group. Clinical signs of the infection are more evident in communities having a high average infection rate and the older people are the principal sufferers. Except amongst the Kisii tribe, the incidence of infection is significantly higher in males than in females.

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* Studies on the Helminth Parasites of Birds in Hyderabad State.

Nematoda III.

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Family : Spiruridae Oerley, 1885.
Subfamily : Spirurinae Railliet, 1915.
Genus : Hadjelia Seurat, 1916.
Hadjelia truncata (Creplin, 1825) Gendre, 1921.

This species was collected on several occasions from the Indian Roller, *Coracias benghalensis*, and it was also obtained once from the Hoopoe, *Upupa epops*. The worms occur under the horny layer of the gizzard and in live condition appear red in colour. The body is cylindrical with slightly attenuated head and tail ends, the anterior extremity being markedly narrower in the male than in the female. The cuticle is thick and carries fine transverse striations. The mouth is bounded by a pair of lateral lips, each consisting of a broad median portion and two small lateral lobes. Each lip is provided with a pair of transparent cuticular caps obliquely set and overlying the lateral lobes. Running round the body at the base of the lips is the cuticular collar or " cadre " of Seurat composed of 8 lobes. Of these the dorso-ventral and the lateral lobes are well developed, whilst the remaining 4, 2 subdorsals and 2 subventrals, are small and inconspicuous. The cephalic papillae are located in the collar and comprise three pairs, two submedian and one lateral. The mouth opens into a cylindrical buccal capsule leading posteriorly into a club-shaped oesophagus. The latter is composed of a short anterior muscular and a relatively longer posterior glandular portion. The intestine runs straight backwards, communicating at the posterior end with the narrow rectum.

Female : The females vary in length from 13 to 16.2 mm. in length with a maximum width of .16–.19 mm. The nerve-ring crosses the oesophagus at about the middle of the muscular portion and is .242 to .257 mm. from the head end. The buccal capsule is 42–52μ long and

*Part of a thesis approved by the University of London for the award of the Ph.D. Degree.

the two portions of the oesophagus measure .44–.47 mm. and 2.32–2.35 mm. in length respectively. The short and bluntly pointed tail is 150 to 154μ long. The conspicuous vulva opens on the ventral surface of the body in the oesophageal region, 2.1–2.13 mm. from the anterior end. The vagina is about 9.5 mm. long. It runs posteriorly from the vulva and then gives origin to the paired uteri. The eggs are oval with thick shells; those in the vagina measure 52μ long by 34μ wide and are embryonated.

Male: The males, which are half as long as the females, measure 7–8 mm. in length and .13–.15 mm. in greatest width. The nerve-ring is .232–.248 mm. from the anterior end. The buccal capsule is 40–50μ long and the two portions of the oesophagus measure .43–.45 and 1.94–2.19 mm. in length respectively. There is a single testis extending forwards to the front end of the oesophagus, where it is reflexed to run posteriorly as far backwards as the middle of the body. The tail is short and bluntly pointed, measuring 103–105μ in length. It is provided with a pair of well-developed lateral alae. There are 6 pairs of pedunculated caudal papillae, of which 4 pairs are preanal and 2 pairs postanal. The spicules are extremely unequal, the ratio between their lengths being 1 : 6. The two spicules measure 1.45–1.55 mm. and .25–.26 mm. in length.

This species has so far been recorded from Europe. It is here reported for the first time from birds in India. The various measurements of the body agree with those given by previous authors except in the fact that eggs here described are considerably larger in size.

Host: *Coracias benghalensis* and *Upupa epops*.

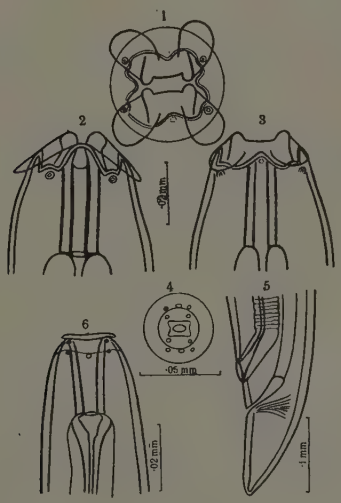
Habitat: Gizzard.

Locality: Hyderabad Deccan (The Nizam's Dominions, India).

Hadjelia inermis (Gedoelst, 1919) Gendre, 1922.

A male and a female specimen belonging to this species were obtained from the gizzard of the white breasted king-fisher, *Halcyon smyrnensis*. The body in both sexes is cylindrical and slightly attenuated at the extremities. The cuticle carries fine transverse striations, which are 7μ apart in the male and 8μ in the female. The mouth is bounded by a pair of trilobed lateral lips and the cuticular collar has 6 lobes. The mouth opens into a cylindrical buccal capsule leading posteriorly into the oesophagus. The latter is differentiated into 2 parts, an anterior muscular and a posterior glandular portion. The nerve-ring surrounds the muscular portion of the oesophagus and is .203 mm. from the anterior end in the female.

Female: The female is 8.25 mm. long and has a maximum transverse diameter of .159 mm. The buccal cavity measures 50μ in length and the constituent portions of the oesophagus are .396 and 1.864 mm. long respectively. The tail is short and bluntly pointed, measuring 95μ in length. The vulva, which is slightly in advance of the junction between oesophagus and intestine, is 1.86 mm. from the anterior end and takes the form of a transverse slit with very



Hadjelia truncata.

Fig. 1. End-on view of head. *Fig.* 2. Head, ventral view. *Fig.* 3. Head, lateral view.

Viguiera euryoptera.

Fig. 4. End-on view of head. *Fig.* 5. Posterior end, female, lateral view. *Fig.* 6. Anterior end, female, lateral view.

prominent cuticular lips, which are subdivided into small nipple-like processes. The vagina runs posteriorly from the vulva. The eggs in the vagina measure 18μ by 13μ. They are unsegmented and are apparently unfertilised.

Male: The male measures 6.9 mm. in length and .15 mm. in greatest thickness. The buccal capsule is 41μ long and the total length of the oesophagus is 2.6 mm. The tail is bluntly pointed, measuring 105μ in length. The caudal alae are well developed and are almost equal

in length. There are six pairs of long pedunculated papillae, out of which 4 pairs are preanal and 2 pairs postanal. In addition a pair of inconspicuous papillae is present near the tip of the tail. The spicules are extremely unequal in length, the left being slightly less than six times as long as the right.

This species has been recorded from a variety of hosts in Africa. It is here described for the first time from India.

Host: *Halcyon smyrnensis.*
Habitat: Gizzard.
Locality: Hyderabad Deccan (The Nizam's Dominions, India).

Family: *Ancyracanthidae* Railliet, 1916.
Subfamily: *Schistorophinae* Travassos, 1918.
Genus: Viguiera Seurat, 1913.
Viguiera euryoptera (Rudolphi, 1819) Seurat, 1913.

This species was recovered on several occasions from the white-bellied drongo, *Dicrurus coerulescens*, and also twice from the bay-backed shrike, *Lanius vittatus*. The worms occur under the horny layer of the gizzard.

These are slender worms with sharply attenuated anterior extremities. The cuticle is thin and carries fine transverse striations which are 22μ apart in the female and 17μ in the male. The nerve-ring surrounds the muscular portion of the oesophagus and is .263 mm. from the anterior end. There are no lips but the head is covered by a disc-like structure, the outer margin of which projects slightly from the underlying part. The cephalic papillae comprise 3 pairs, 2 pairs being submedian and one pair lateral. The mouth opens into a cylindrical buccal capsule leading posteriorly into the oesophagus. The latter is divided into a narrow muscular anterior portion and a broader, glandular posterior portion.

Female: The females are two to three times as long as the males, measuring 6–11 mm. in length and .251–.273 mm. in maximum width. The buccal capsule is 30μ long and the length measurements of the two portions of the oesophagus are .42 and 3.1 mm. respectively, while the entire organ occupies about $\frac{1}{3}$ of the body length. The tail is short and conical, the distance from the anus to the tip of the tail being 102–103μ. The vulva is a large circular aperture lying 35μ in front of the anal aperture. The vagina has a thick muscular coating and a chitinous lining. It is a long tube running forwards from the vulva to divide into the paired uteri. The eggs are thick shelled and

embryonated, measuring 38μ long by 21μ wide.

Male: The males are 3.5–4.5 mm. in length and have a maximum transverse diameter of .204 mm. The buccal capsule measures 28μ in length and the oesophagus is 1.53 mm. long. The muscular portion of the latter occupies .29 mm. As the tail is spirally twisted and provided with asymmetrical alae, it is difficult to mount it for a ventral view. The distance from the cloaca to the tip of the tail is 410μ. The caudal papillae are pedunculated and consist of 9 pairs of preanals and 2 pairs of postanals. Seurat (1913) while describing 9 pairs, figures only 7 preanals on the right and 9 on the left. In all specimens examined by the writer the number is invariably 9 on each side. The spicules are very unequal, measuring .194 and 1.136 mm. in length respectively.

Seurat gave a detailed description of Rudolphi's "*Spiroptera*" *euryoptera*, creating a new genus *Viguiera* for its reception. This species has been recorded from various parts of Europe and it is here reported for the first time in India and also from a new host.

Host: *Lanius vittatus* and *Dicrurus coerulescens*.

Habitat: Gizzard.

Locality: Hyderabad Deccan (The Nizam's Dominions, India).

Genus: SCHISTOROPHUS Railliet, 1916.
(*Syn:* *Quasithelazia* Maplestone, 1932).
Schistorophus tenuis (Maplestone, 1932).
(*Syn.:* *Quasithelazia tenuis* Maplestone, 1932).

On numerous occasions this parasite was recovered from the gizzard of the white-breasted king-fisher, *Halcyon smyrnensis*. The worms lie under the horny layer of the gizzard.

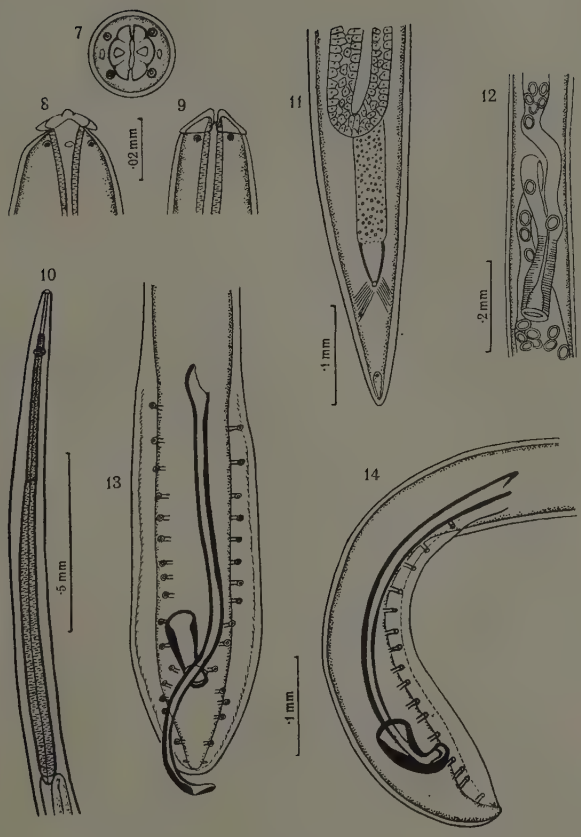
The body of the worm is long and slender and in both sexes it gradually tapers towards the anterior extremity. The females are from two to three times as long as the males. The cuticle is smooth and finely striated transversely. The striations are set at intervals of 11μ in the female and 9μ in the male. The head carries 2 lateral triangular lips indistinctly divided into 3 lobes. Each lip has a cuticular covering which projects slightly dorso-ventrally. The cephalic papillae comprise 3 pairs, two pairs submedian and one pair lateral. The nerve-ring surrounds the oesophagus near its junction with the buccal capsule and it is 166μ from the anterior end in the female and 120μ in the male. The mouth leads into a long buccal capsule with striated walls. In lateral view its anterior end is slightly expanded but in dorso-ventral aspect it has a uniform thickness. It measures 100–125μ in the female and 95μ in the male. The oesophagus is

composed of a short anterior muscular and a long posterior glandular portion, the former being nearly half as long as the latter. In the female, the two portions measure .407 and .82 mm. and in the male .39 and .81 mm. in length respectively. The intestine runs in a straight course towards the posterior extremity and in the female it communicates with the exterior by a short rectum.

Female: The females measure 18.6–20.5 mm. in length with a maximum thickness of .123 mm. In the region of the oesophagus the body is narrowed towards the front end, whereas the posterior extremity is relatively much broader and forms a bluntly pointed tail. The distance from the anus to the tail is 109–120μ. The vulva is flush with the body surface at a distance of 10.7 mm. from the anterior end. The vagina is directed anteriorly. It is a short tube dividing into the paired uteri. The latter are divergent; the anterior one runs in a straight course, whereas the posterior one proceeds forwards only for a short distance and then curves back to run posteriorly. The anterior ovary extends quite close to the hind end of the oesophagus and the posterior one stretches backwards to the vicinity of the rectum, where it is reflexed for a considerable distance. The eggs in the uteri measured 30μ long by 22μ wide. They are thick-shelled and embryonated.

Male: The male varies in length from 7.5–8.5 mm. and has a maximum transverse diameter of 110μ. The anterior end is more attenuated in the male than in the female. The posterior extremity is flexed ventrally and forms a bluntly pointed tail. It is furnished with well-developed caudal alae about 420μ long. The cloacal aperture lies at a distance of .09 mm. from the tip of the tail. There are present numerous pedunculated caudal papillae supporting the caudal alae. The preanals vary in number from 11 to 14 pairs. While most of them are disposed symmetrically, some of the anterior ones may be asymmetrical. In the tail (Fig. 13), there are 12 papillae on the right side and 11 on the left. The number of postanal papillae is constant, there being always 4 pairs posterior to the cloaca. In addition to these a pair of small papillae is present near the tip of the tail. The spicules are dissimilar in shape and unequal in length, the left being 5 times as long as the right. They measure 424–473μ and 89–95μ in length respectively. The left spicule is slender and tubular and the right one is short, stout and trough-like.

Discussion: Maplestone (1932) created a new genus *Quasithelazia* to accommodate this worm. His observations were based on a single male specimen. The writer, who had the opportunity of examining



Schistorophus tenuis.

Fig. 7. End-on view of head. *Fig.* 8. Head, lateral view. *Fig.* 9. Head, ventral view. *Fig.* 10. Anterior end, female, lateral view. *Fig.* 11. Posterior end, female, ventral view. *Fig.* 12. Body of female, showing vulva, vagina, origin of uteri. *Fig.* 13. Posterior end, male, ventral view. *Fig.* 14. Posterior end, male, lateral view.

on different occasions, ample material consisting of both females and males, finds that there are sound reasons for considering the genus *Quasithelazia* Maplestone to be identical with the genus *Schistorophus* Railliet, 1916. In his account of the male worm Maplestone states, " between the vestibule and the oesophagus there is a swollen, slightly lobulate structure, which is probably glandular in function." Even after prolonged search the writer was unable to locate this structure. This is, however, the position of the nerve-ring which, as mentioned above, encircles the anterior portion of the oesophagus, near its junction with the vestibule.

The structure of the lips is similar to that found in various species of the genus *Schistorophus* Railliet, 1916, there being two conical lips with cuticular caps projecting slightly dorsally and ventrally. In possessing a long vestibule and a divided oesophagus, it again resembles *Schistorophus*. The presence of numerous preanal papillae does not necessarily show the affinity of the worm to the subfamily *Thelaziinae* Baylis and Daubney, 1926, since it is a typical character of *Schistorophus* species. The size of the spicules and the disposition of the female reproductive organs also point towards the same conclusion.

The worm under discussion differs from other known species of *Schistorophus* in that the cuticular caps on the head are very rudimentary and the number and disposition of the caudal papillae in the male also differ from other species.

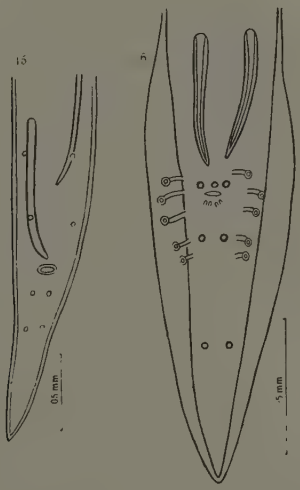
Host : Halcyon smyrnensis.
Habitat : Under the horny layer of the gizzard.
Locality : Hyderabad Deccan (The Nizam's Dominions, India.)

Family : Tetrameridae Travassos, 1914.
Subfamily : Tetramerinae Railliet, 1915.
Genus : Microtetrameres Travassos, 1915.
Microtetrameres inermis (Linstow, 1879) Travassos, 1915.

This parasite was obtained on several occasions from the Brahminy Myna, *Temenuchus pagodarum* and also once from *Brachypternus bengalensis*. The females are found in the glands of the proventriculus while the males lie freely in the lumen of the organ. The worms exhibit sexual dimorphism in common with other species belonging to the genus *Microtetrameres*, the males being filiform and the females fusiform and spirally coiled.

Female : The females appear blood red in colour and form cysts measuring about 1.5–2 mm. The spirally coiled body is about 4.6–5 mm. long and has a maximum transverse diameter of .472 mm. The head

and the tail ends are sharply attenuated while the maximum thickness is reached towards the middle of the body. The cuticle of the anterior end is thrown into wrinkles. The flask-shaped buccal cavity measures 20μ long. The oesophagus is divided into muscular and glandular portions. The nerve-ring surrounds the glundular portion at about the middle of its length. The finely pointed tail measures 133μ in length. The vulva is inconspicuous and is located .1–.14 mm. anterior to the



Microtetrameres inermis.

Fig. 15. Posterior end, male, ventral view.

Physaloptera alata.

Fig. 16. Posterior end, male, ventral view.

anal-opening. The vagina runs anteriorly from the vulva. The eggs are cylindrical and are operculated at each end. They are 44–53μ long and 26–37μ wide, and are embryonated.

Male: The males are very slender and smaller than the females. They vary in length from 1.38–2.16 mm. and have a maximum width of .054–2.06 mm. The buccal capsule measures 14μ in length. The total length of the oesophagus varies from .513–.536 mm. whilst the anterior muscular portion is 167–180μ long. The tail measures 92–112μ

in length. The cloaca has a cuticular rim projecting on the ventral surface of the body. The caudal alae are lacking, and the caudal papillae are somewhat asymmetrically arranged. There are two pairs preanal and three pairs postanal in position. The spicules are extremely unequal in length, the long spicule extending into the oesophageal region of the body. They measure 1.78–1.84 mm. and .087–.096 mm. in length respectively.

The measurements of the body tally closely with those given by Seurat (1913). This species is here recorded for the first time from India and also from two new hosts.

Host: *Temenuchus pagodarum* and *Brachypternus bengalensis.*
Habitat: Proventriculus.
Locality: Hyderabad Deccan (The Nizam's Dominions, India).

Family: PHYSALOPTERIDAE Leiper, 1908.
Subfamily: PHYSALOPTERINAE Railliet, 1893.
Genus: PHYSALOPTERA Rudolphi, 1819.
Physaloptera alata Rudolphi, 1819.

This species occurred once in the oesophagus of a sparrow hawk, *Accipiter nisus.* The material consists of a male, a female and a fragment.

These are stout bodied worms having a thick cuticle which is transversely striated. The extremities in both sexes are slightly attenuated. The mouth is bounded by a pair of well developed lateral lips, each possessing a large triangular external tooth and three smaller internal teeth. The cervical cuticle forms a collar round the anterior end. The cervical papillae are conspicuous, situated at the base of the cuticular collar and the excretory pore opens slightly posterior to the latter on the ventral surface. The oesophagus is club-shaped and divided into two portions.

Female: The female is 18.5 mm. long and has a maximum thickness of .872 mm. The nerve-ring, cervical papillae and excretory pore are .4 mm., .5 mm., and .7 mm. respectively from the anterior end. The muscular portion of the oesophagus measures .5 mm. and the glandular portion 3.437 mm. the total length being slightly less than $\frac{1}{5}$ of the body length. The conical tail is .64 mm. long and occupies about $\frac{1}{29}$ of the body length. The vulva is flush with the body surface, 3.187 mm. from the anterior end. The vagina is a long muscular tube running backwards to divide into the paired uteri. The ovaries are located in the posterior portion of the body. The worm under observation is immature since no ova could be found in the genital ducts.

Male: The male measures 15 mm. in length and .674 mm. in greatest width. The nerve-ring, cervical papillae and excretory pore are .28 mm., .48 mm., and .7 mm., respectively from the anterior end. The two portions of the oesophagus measure .312–.343 and 2.65–2.81 mm. in length respectively, the entire organ thus occupying about $\frac{1}{5}$ of the body length. From a slight distance in front of the cloaca the body narrows gradually to end in a pointed tip. The distance from the cloaca to the tip of the tail is 1.05 mm. The well developed caudal alae are about 1.57 mm. long and there are 5 pairs of pedunculated papillae. Of the sessile papillae there are 3 in front of the cloaca and 2 pairs immediately posterior to it. Further down the tail there are 2 additional pairs of sessile papillae. The spicules are slender and equal, measuring .494 mm. in length. They have pointed tips.

This species was recorded by Baylis and Daubney (1922) from *Circus pygarus* in India. The worm here described was collected from the type host, the sparrow hawk, in Hyderabad Deccan.

Host: Accipiter nisus.

Habitat: Oesophagus.

Locality: Hyderabad Deccan (The Nizam's Dominions, India).

Family: Oxyuridae Cobbold, 1864.

Subfamily: Cosmocercinae Railliet, 1916.

Genus: Syphaciella Monnig, 1924.

Syphaciella indica Maplestone, 1931.

Numerous specimens of this species were collected on several occasions from the caecum and the intestine of the common Indian sand grouse, *Pterocles exustus.*

These are rather small worms with the body in both sexes tapering towards the extremities. The head is provided with a distinct cephalic inflation of the cuticle which is followed by the lateral flanges, and in the male these flanges are continuous with the caudal alae. The cuticle is smooth and unstriated. The head bears three bilobed lips. The dorsal lip carries a pair of cephalic papillae, while each of the subventrals has a single large papilla. The oesophagus consists of a cylindrical corpus which is separated by a constriction from a posterior globular bulb. At its anterior end it is provided with two pairs of finely pointed teeth-like structures. At its anterior termination, the intestine presents the appearance of an inverted flask.

Female: The female is 5.5–7 mm. long and 250–290μ in greatest width. The cephalic inflation measures 110–113μ in length. The total length of the oesophagus is .447–.51 mm., the bulb measuring

90–102μ in length. The body tapers gradually towards the posterior extremity and forms a long pointed tail measuring .77–.88 mm. in length. The vulva is very prominent, being surrounded by a distinct cuticular fringe. It is 1.36–1.39 mm. from the anterior end and opens into a posteriorly directed long vagina which is connected to a globular sphincter. This is followed by the common trunk of the uterus which enlarges to form a long sac-like reservoir extending far backwards to the anal region of the body. Here it gives origin to the two uteri which immediately bend forwards running parallel to each other, and at their terminal portions form globular swellings, the seminal receptacles. The oviducts are small narrow ducts joined to the ovaries. The latter are long filamentous bodies extending far forwards terminating near the posterior end of the oesophagus. The thick-walled oval eggs are flattened on one side and are operculated at one pole. They are 103μ long and 45μ wide.

Male: The male, which is smaller and more slender than the female, measures 4.5–4.6 mm. in length and its greatest diameter is 185–195μ. The cuticular inflation surrounding the anterior end extends from 100–110μ in length. The total length of the oesophagus, including the bulb is 410μ, the latter being 75μ long. The posterior extremity tapers to a spike-like point. Posterior to the cloaca it is marked with a distinct furrow on its ventral surface, and it is also provided with a pair of well developed caudal alae which, as noted above, are continuous in front with the lateral flanges of the body and are posteriorly marked with fine transverse striae on the ventral surface. The distance from the cloaca to the tip of the tail is 230μ. There are 4 pairs of caudal papillae, 2 pairs being preanal and two postanal in position. The former, which are situated close together in front of the cloacal aperture, are more developed than the latter, the posterior pair of which is sometimes difficult to observe. The spicules are 95μ long and lightly chitinized. Their heads are rounded, whilst their distal ends are finely pointed. An accessory piece is present which, unlike the spicules, is heavily chitinized.

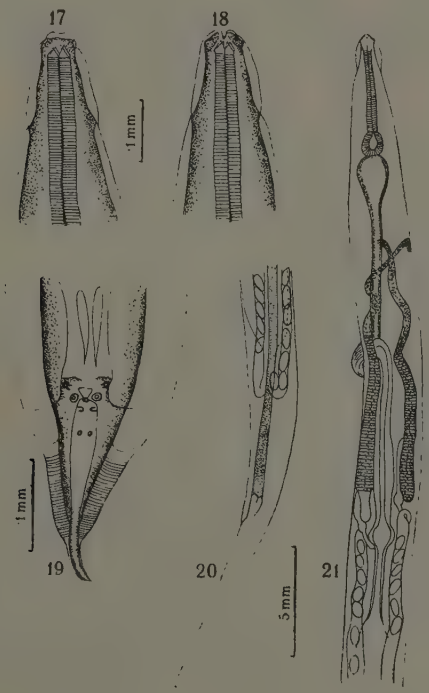
Discussion: The genus *Syphaciella* was created by Mönnig (1924) with *S. capensis* as its genotype. Maplestone (1931) discovered the second species, *S. indica* from a sand grouse, *Pterocles exustus*, which had died in the Calcutta Zoological Gardens. This species was again recorded by Akhtar (1939) from the same host in Afghanistan. It is here re-described from material obtained in Hyderabad and a detailed account of the female genitalia is given. As stated by Maplestone, *S. indica* differs from *S. capensis* in possessing a distinct cephalic

cuticular swelling, and also differs in the number and arrangement of the caudal papillae in the male. Here it may be mentioned that the male of this species is further distinguished by the presence of fine transverse striae on the ventral surface of the posterior half of the caudal alae. As regards other general characters there is a close resemblance between the two species as pointed out by Maplestone.

Host : Pterocles exustus.

Habitat : Caecum and intestine.

Locality : Hyderabad Deccan (The Nizam's Dominions, India).



Syphaciella indica.

Fig. 17. Anterior end, dorsal view. *Fig.* 18. Anterior end, ventral view. *Fig.* 19. Posterior end, male, ventral view. *Fig.* 20. Posterior end, female, lateral view. *Fig.* 21. Anterior portion of body, female, showing alimentary canal and the reproductive organs.

SUMMARY.

1. *Hadjelia truncata, Hadjelia inermis* and *Viguiera euryoptera* are newly recorded from India.

2. *Quasithelazia tenuis* is described in detail and the genus *Quasithelazia* Maplestone is considered to be a synonym of the genus *Schistorophus* Railliet, 1916.

3. *Microtetrameres inermis* is newly recorded from India and is described from two new hosts, *Temenuchus pagodarum* and *Brachypternus bengalensis*.

4. *Physaloptera alata* is described from the type-host, *Accipiter nisus*.

5. *Syphaciella indica* is described in detail and compared with the original description.

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* Studies on the Helminth Parasites of Birds in Hyderabad State.

Nematoda IV.

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Family: Filariidae (Cobbold, 1864) Claus, 1885.

Subfamily: Aproctinae Yorke & Maplestone, 1926.

Buckleyfilaria buckleyi n.g., n.sp.

Numerous male and female specimens of this worm were collected from the body cavity of " Dayal," *Copsychus saularis*. In live condition the entire body surface is covered with minute cuticular papillae but it is difficult to observe them in fixed and preserved specimens.

These are medium sized worms having slightly tapering extremities. In the males, which are about half as long as the females, the body is straight except for the tail which is spirally twisted. The cuticle is unstriated and in both sexes the head and tail ends are bluntly rounded. The nerve-ring surrounds the oesophagus at about one-fifth of its length from the anterior end and immediately posterior to it is the excretory vesicle which opens on the ventral surface of the body by means of a narrow excretory duct. The head is truncated with rounded sides, and is without lips or epaulette-like ornamentations. The cephalic papillae are easily distinguishable and are arranged in two circles, the inner circle comprising a pair of lateral and two pairs of sub-median papillae, while in the outer circle there are only two pairs of sub-medians. The mouth communicates by means of a short thin walled vestibule with the oesophagus which is club-shaped and undivided. The oesophago-intestinal valves are fairly well-developed and consist of three lobes extending into the lumen of the intestine. The latter is as thick as the oesophagus.

Female: The females vary from 26–31 mm. in length and from .66–.82 mm. in maximum transverse diameter. The vestibule is 21μ long by 29μ wide. The nerve-ring is 131μ and the excretory pore 233μ from the anterior end. The oesophagus measures .7–.74 mm. in length.

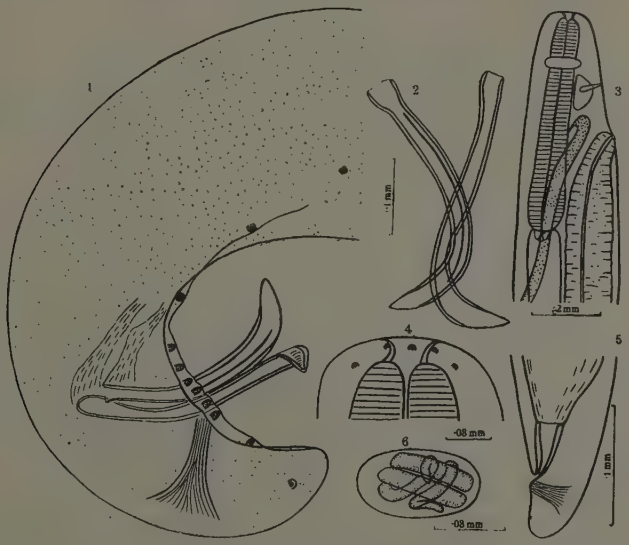
* Part of a thesis approved by the University of London for the award of the Ph.D. Degree.

The tail is short and bluntly rounded and the anus is 191–205μ from the tip of the tail. The vulva is oesophageal in position and is located on a slight prominence of the body wall on the ventral surface, .36–.42 mm. from the anterior end. The vagina, which runs backwards from the vulva, has thick muscular walls and a cuticular lining. It is separated by a slight constriction from a much wider tube, the common trunk of the uterus. At its posterior end the trunk is narrowed and reflexed for a short distance before it turns backwards once again to be divided into the paired uteri. The ovaries are long, flattened, ribbon-like bodies lying one behind the other in the post-oesophageal portion of the body. The thin-shelled egg is 53μ long by 30μ wide and contains a much-coiled embryo.

Male: The males are slender bodied, measuring 7.8–16.04 mm. in length and .46–.54 mm. in greatest width. The oesophagus is .54–.62 mm. long. The distance from the head end to the nerve-ring is 127μ and to the excretory pore 203μ. There is a single gonad and the vas deferens is a long tube joined anteriorly to the narrow testis. The latter extends into the region of the oesophagus where turning backwards, it forms a twisted loop and ends in a posteriorly directed tip, not far distant from the front end of the intestine. The posterior extremity of the body is spirally twisted and ends in a short ventrally flexed tail, having a bluntly rounded tip. There is a pair of lateral alae extending to a considerable extent in front of the cloaca. The caudal papillae consist of 11 pairs. The first three pairs are separated by wider intervals than the next 6 pairs which are quite close to each other. Of the latter, 2 pairs are preanal in position, probably 3 pairs adanal and one pair postanal. Further back there is another pair of postanal papillae and from here half way down to the tip of the tail is the last pair of postanal papillae situated in the lateral fields. The spicules are subequal, stout and strongly chitinized ; they frequently project outwards through the cloacal aperture which they distend considerably. The body of both spicules is of uniform thickness with slightly expanded heads and pointed tips. They measure 386–422μ in length.

Discussion: This worm bears close affinity to the genus *Pelecitus* Railliet and Henry, 1910, in possessing caudal alae in the male, a feature of diagonistic value in the sub-family Aproctinae Yorke and Maplestone, 1926. But the lateral flanges along the whole length of the body, which are so characteristic of *Pelecitus*, are lacking in the present worm. The spicules which are very short and delicate in all the species belonging to the genus *Pelecitus*, are very stout and strongly chitinized

in this worm. Further there is a difference in the habitat in the host, for the members of the genus *Pelecitus* are mostly parasites of the muscles and tendons of the legs of birds, whereas the new worm is found in the body cavity of a Passerine bird. Again the minute papillae on the general surface of the body and the presence of buccal cavity and strongly chitinized spicules in the male suggest affinity with the genus *Squamofilaria* Schmerling, 1925 (syn : *Coronofilaria* Yorke and Maplestone, 1926). But the worm under discussion differs from that genus in possessing in the male, well-developed caudal alae and numerous caudal papillae. In view of the above differences, the writer considers it necessary to create a new genus for this worm. It is proposed to name it *Buckleyfilaria buckleyi* n.g., n.sp., after Professor J. J. C. Buckley.



Buckleyfilaria buckleyi n.g., n.sp.

Fig. 1. Posterior end, male, lateral view. *Fig.* 2. Spicules. *Fig.* 3. Anterior end, female, lateral view. *Fig.* 4. Head, lateral view. *Fig.* 5. Posterior end, female, lateral view. *Fig.* 6. Egg.

Generic diagnosis : Medium sized worms with rounded head and tail ends ; mouth without lips ; cephalic papillae, four pairs submedian and one pair lateral ; cuticle devoid of transverse striations ; lateral

flanges absent; buccal cavity present; oesophagus undivided, cylindrical, and club-shaped. *Female:* Vulva oesophageal in position; eggs with coiled embryos. *Male:* Tail spirally twisted, provided with a pair of lateral alae; caudal papillae numerous, about 11 pairs, 5 pairs preanal, 3 pairs adanal and 3 pairs post anal; spicules subequal, stout and strongly chitinized. Parasites of the body cavity of birds.

Type species: Buckleyfilaria buckleyi.

Host: Copsychus saularis.

Habitat: Body cavity.

Locality: Hyderabad Deccan (The Nizam's Dominions, India).

Type specimens are deposited in the museum of the Department of Parasitology, London School of Hygiene and Tropical Medicine.

Genus: EUFILARIA Seurat, 1921.

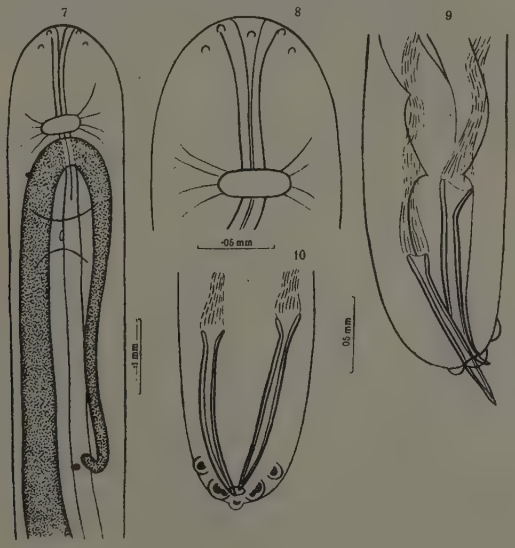
Eufilaria asiatica n.sp.

This species was recovered twice from the neck of the common crow, *Corvus splendens*. On both occasions the worms were found underneath the skin, alongside the trachea. The material consists of three male specimens only.

The body of the male worm is straight and is of almost uniform diameter. It is rounded at the extremities with the head presenting a slightly swollen appearance. The cuticle is thin and the internal organs were easily discernible in the live specimens. The transverse cuticular striae are very fine and could be observed only under high magnification. They are very closely set, being not more than 1μ apart. The lateral fields are very broad measuring 21μ in width. The mouth is devoid of lips and the cephalic papillae comprise 3 pairs, of which 2 pairs are sub-median, and one pair lateral. Cervical papillae were not observed. The nerve-ring encircles the oesophagus at about the middle of its length and is 100–120μ from the anterior end. The excretory pore is quite prominent and is situated on the ventral surface of the body behind the junction of the oesophagus and the intestine. The distance from the excretory pore to the head end is .273 mm. The mouth leads into the oesophagus which measures .233 mm. It is undivided and in the form of a thin narrow tube, a feature so characteristic of the genus *Eufilaria*. The junction between the oesophagus and the intestine is not well defined, the oesophago-intestinal valves being absent.

Male: The males measure 8.904–10.285 mm. with a maximum transverse diameter of 115–125μ. There is a single testis extending into the region of the oesophagus; it is reflexed immediately posterior

to the nerve-ring and runs backwards for a considerable distance before terminating in an anteriorly directed tip. The cloacal aperture is sub-terminal and the tail, as mentioned above, is uncoiled and rounded ; it is devoid of caudal alae. The caudal papillae, however, are prominent, one pair being preanal and one pair adanal. Of these, the adanal papillae are larger in size and represent fused papillae, each with a double pulp and a common cuticular cap. In addition to the paired papillae there is also present a single median postanal papilla which projects posteriorly at the tip of the tail. The spicules are similar and subequal in length ; they are dagger-shaped with expanded heads and pointed tips. In one specimen they measured 113 and 119μ and in another 114 and 124μ respectively.



Eufilaria asiatica n.sp.
Fig. 7. Anterior end, male, ventral view. *Fig.* 8. Head, male, lateral view. *Fig.* 9. Posterior end, male, lateral view. *Fig.* 10. Posterior end, male, ventral view.

Discussion : This worm belongs to the genus *Eufilaria* Seurat, 1921, distinguishing features of which are the undivided narrow and transparent oesophagus, the straight and rounded extremities, the male with subequal spicules and devoid of caudal alae. The type species was described by Seurat (1921) from *Passer hispaniolensis*.

Filaria capsulata Annett, Dutton and Elliott, 1901, has been referred to this genus by Yorke and Maplestone, 1926. The systematic position of this species is rather doubtful as it is not fully described. Yamaguti (1935) described *E. lari* of which the female only is known. The worm under discussion differs from all the known species of *Eufilaria* in that the male has well developed caudal papillae. Instead of creating a new genus for the present species, the writer proposes to modify Seurat's definition of *Eufilaria* to the extent that cephalic papillae may be present or absent, and in the male, caudal papillae may be present or lacking.

Von Linstow (1904) described "*Filaria*" *vivipara* from the peritoneum of *Corvus splendens* in Ceylon. As the description of this species is meagre and based on females only, it is not possible to ascertain its relationship with the present worm, which is also parasitic in the same host, the common crow.

Host: *Corvus splendens.*

Habitat: Subcutaneous connective tissue.

Locality: Hyderabad Deccan (The Nizam's Dominions, India).

Lerouxinema lerouxi n.g., n.sp.

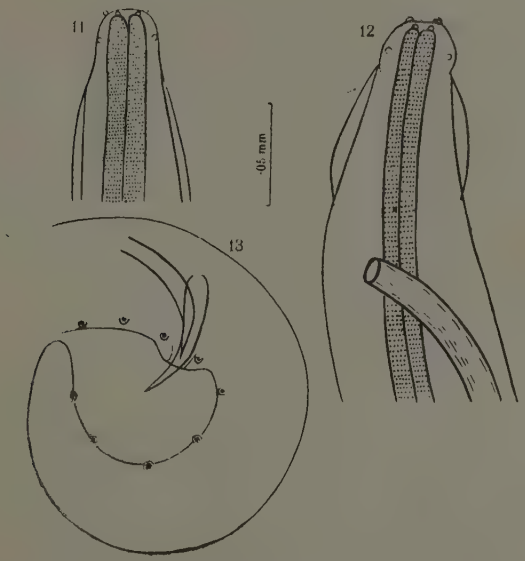
Three specimens of this species, two males and a single female, were obtained once from the heart of the red spur-fowl, *Galloperdix spadicea.*

These worms have very slender bodies, the maximum transverse diameter not exceeding 67μ in the female and 45μ in the male. The female is considerably larger in size than the male. The body is cylindrical in shape with bluntly pointed head and tail. In both sexes the head is set off from the rest of the body by a slight constriction. The cuticle is thin and unstriated. Cephalic alae are present, being more marked in the female than in the male. The head is without lips or cuticular ornamentations of any kind but the cephalic papillae are distinct, there being 4 pairs of submedian and an additional pair of laterally situated papillae at the base of the head. The mouth leads by an extremely short vestibule into the oesophagus which is club shaped and undivided.

Female: The single female specimen available for study is so much coiled upon itself that it is not possible to determine its exact length; it is about 15 mm. long. The maximum width of the body is 67μ and the diameter of the knob-like head is 17.7μ. The vulva opens in the oesophageal region of the body, 58μ from the anterior end and at this level the thickness of the body is 38μ. The vagina runs backwards from

the vulva. The eggs are very small with a thin membranous egg shell, measuring 9–12μ long by 3–4μ wide.

Male: The males measure 10.28–10.52 mm. in length and have a maximum thickness of 45μ at about the middle of the body. The oesophagus is 0.63 mm. long and the nerve-ring surrounds it at a distance of 97μ from the anterior end. The posterior extremity is coiled in 2 or 3 whorls. The digitiform tail is strongly flexed ventrally



Lerouxinema lerouxi n.g., n.sp.

Fig. 11. Anterior end, male, ventral view. *Fig.* 12. Anterior end, female, ventral view. *Fig.* 13. Posterior end, male, lateral view.

and the cloacal aperture is on a slight protubrance of the body wall situated 110–115μ from the tip of the tail. Caudal alae are absent but the caudal papillae are distinct and symmetrically arranged on the ventral surface of the body. They comprise 9 pairs, out of which 3 pairs are preanal and the remaining 6 pairs postanal. There are two short subequal spicules with very finely pointed tips ; they measure 33 and 39μ long respectively and have a maximum thickness of 5–6μ.

Discussion: This worm belongs to the subfamily Aproctinae Yorke and Maplestone, 1926, as it is without any cuticular ornamentations on the head and has subequal spicules in the male, with the vulva in the oesophageal region in the female. Many genera of nematodes belonging to this subfamily have been described from the vascular system of birds, viz. *Splendidofilaria* Skrjabin, 1923; *Chandlerella* Yorke and Maplestone, 1926; *Cardiofilaria* Strom, 1937; *Vagrifilaria* Augustine, 1937 and *Bhalfilaria* Bhalerao and Rao, 1944. The worm described herein cannot be accommodated in any of these known genera and presents certain marked differences. It can be distinguished from *Splendidofilaria* by the absence of the cuticular bosses on the general body surface. As regards *Chandlerella*, the writer considers the validity of this genus as doubtful. The type species *C. bosei* (Chandler, 1924) Yorke and Maplestone, 1926, resembles very closely the genus *Splendidofilaria*, especially in the form of the spicules and the characteristic digitiform tail of the male with inconspicuous caudal papillae. The cuticular bosses, which are a diagnostic feature of *Splendidofilaria*, have not been noted in *C. bosei* by Chandler who states that "the cuticle is smooth and the body white." Pandit, Menon and Iyer (1929) who record this species from the right ventricle of the heart of the crow, have also failed to observe the cuticular bosses. But the writer who has had the opportunity of examining both live and fixed specimens of *Splendidofilaria*, finds that the cuticular bosses are discernible only in the live condition and it is difficult to observe them in preserved material. It therefore appears probable that the genus *Chandlerella* is identical with *Splendidofilaria*. The worm here described from the heart of the red spur-fowl can be further distinguished from the other genera mentioned above, by possessing cephalic alae and by the numerous preanal and postanal papillae on the male tail. Moreover, the body of the worm is extremely slender as compared with all the known species of filariid nematodes so far described from the bird hosts. The writer, therefore, feels justified in establishing a new genus to accommodate this new worm. It is proposed to name it *Lerouxinema lerouxi* n.g., n.sp., after Dr. P. L. Leroux.

Generic diagnosis: Aproctinae with very slender bodies and bluntly pointed head and tail ends; cuticle smooth and devoid of transverse striations or bossess; mouth without lips; cephalic alae and papillae present; oesophagus undivided and club-shaped. *Female:* Vulva in the oesophageal portion of the body; tail bluntly pointed and anus atrophied; eggs thin shelled and embryonated. *Male:* Tail digitiform; caudal alae absent; caudal papillae, three pairs preanal and six pairs

postanal; spicules very small, subequal, with finely pointed tips. Parasites in the heart of galliform birds.

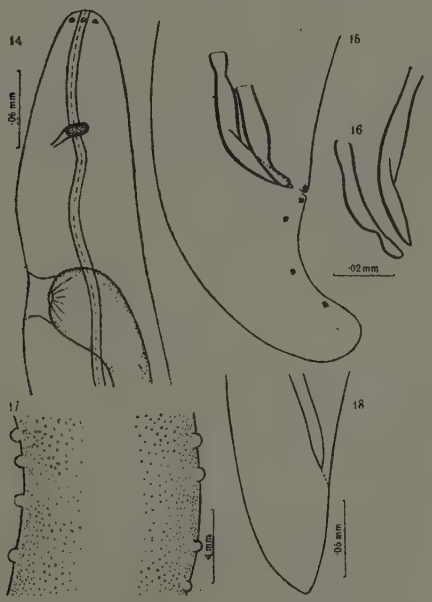
Type species: Lerouxinema lerouxi.

Host: Galloperdix spadicea.

Habitat: Heart blood.

Locality: Hyderabad Deccan (The Nizam's Dominions, India).

Type specimens are deposited in the museum of the Department of Parasitology, London School of Hygiene and Tropical Medicine.



Splendidofilaria brevispiculum n.sp.

Fig. 14. Anterior end, female, lateral view. *Fig.* 15. Posterior end, male, lateral view. *Fig.* 16. Spicules. *Fig.* 17. Body showing cuticular bosses. *Fig.* 18. Posterior end, female, lateral view.

Genus: SPLENDIDOFILARIA Skrjabin, 1923.

Splendidofilaria brevispiculum n.sp.

One male and one female specimen of the species to be described were obtained once from the Indian ring dove, *Streptopelia decaocto* and on another occasion a single male specimen of the same species was

recovered from the white-breasted king-fisher, *Halcyon smyrnensis*. In both hosts the worms occurred in the right ventricle of the heart.

These are rather slender worms with slightly attenuated anterior and posterior ends. The thin and smooth cuticle is devoid of transverse striations but is furnished with bosses, which though easily discernible in the live condition, are difficult to observe in fixed material. The head carries 2 pairs of submedian and one pair of lateral papillae. There is no buccal capsule so that the mouth opens directly into the oesophagus which is a very narrow tube. The nerve-ring encircles the oesophagus at a distance of 73μ from the anterior end in the female.

Female: The single female measures 15.37 mm. in length and has a maximum thickness of 240μ at about the middle of the body. The diameter of the head is 26μ and the oesophagus is .873 mm. long. The vulva is located in the oesophageal region and is sunk in a depression on the ventral surface. The distance from the anterior end to the vulva is .182 mm. The tail forms a bluntly pointed tip. The anus is closed and the rectum is atrophied. The distance from the tip of the tail to the anus is about 70μ. The female is viviparous, the embryos measuring 82μ long by 3μ wide.

Male: The body of the male is more slender and smaller than that of the female. It measures 9.38–10.42 mm. in length and 160μ in maximum thickness. The oesophagus is .631–.74 mm. long. The tail is short and bluntly rounded. The cloacal aperture is 51–60μ from the tip of the tail. The caudal alae are lacking and the caudal papillae are inconspicuous. There are altogether 5 pairs, and out of these one pair is immediately in front of the cloaca and one pair is immediately behind it. The remaining three pairs are equally spaced down the tail. The spicules are short and subequal; in one specimen they measured 37 and 54μ and in the other 44 and 58μ respectively. Both spicules have slightly expanded heads and bluntly pointed tips. The two spicules differ also in shape; whereas the long spicule has a kink at about the middle of its length, the short one has its tip set off from the rest of its body by a short constriction.

Discussion: Skrjabin (1923) established the genus *Splendidofilaria* with *S. pawlowskyi* as the genotype. The description of this species is based on material collected from the heart of *Otomela phaenicuroides*. Travassos (1926) described a second species, *S. gedoelsti* from the inguinal cavity of *Selenidra maculirostris*. The worm here described from Indian birds constitutes a third species. Being considerably smaller in size it differs from *S. gedoelsti* in all body measurements. In the male of *S. gedoelsti* there are 8 pairs of caudal papillae, whereas

there are only 5 in the present worm. When compared with the type species, *S. pawlowskyi*, there is no marked difference in size, but it can be differentiated from it in other respects, especially in the size and shape of the spicules and the number and disposition of the caudal papillae in the male. In *S. pawlowskyi* the spicules are relatively larger and similar in structure, whereas in the worm under discussion they are not only smaller but differ in shape. There are no preanal papillae in *S. pawlowskyi*, but a pair is present immediately in front of the cloaca in the Indian species. The postanal papillae though equal in number, are differently arranged in the two species ; in *S. pawlowskyi* they form a single series on each side, but in the other worm the immediately postanal pair is situated medially and the remaining 3 pairs are relatively external in position. Moreover, the last 3 pairs are equally spaced in the Indian species, whereas the last 2 are close together in *S. pawlowskyi*. In the female of the latter species the tail is broadly rounded but it is somewhat pointed in the present species. It is therefore concluded that the species under consideration is new and it is proposed to name it *S. brevispiculum* n.sp.

Host : Streptopelia decaocto and *Halcyon smyrnensis.*
Habitat : Right ventricle of the heart.
Locality : Hyderabad Deccan (The Nizam's Dominions, India).

Subfamily : Tetracheilonematinae Wehr, 1935.
Genus : Squamofilaria Schmerling, 1925.
Squamofilaria coronata (Rudolphi, 1809) Schmerling, 1925.
(Syn : *Filaria coronata* Rudolphi, 1809).

Four males and three females of this species were recovered once from an Indian Roller, *Coracias benghalensis.* The worms occurred in the neck just underneath the skin.

These are medium sized worms, the females being larger than the males. The body in both sexes is cylindrical and attenuated towards the extremities. The cuticle is thick but without transverse striations. The general body surface is covered with minute cuticular papillae which are more prominent in the male, especially on the ventral surface of the post extremity. The nerve-ring surrounds the oesophagus near its anterior end and the excretory pore is posterior to it. There are no definite lips but the mouth is surrounded by a circumoral cuticular ring or collar. Cephalic papillae are distinct and consist of 4 pairs of submedian and one pair of lateral papillae. The mouth opens into a vestibule with thick walls which diverge anteriorly so as to form a

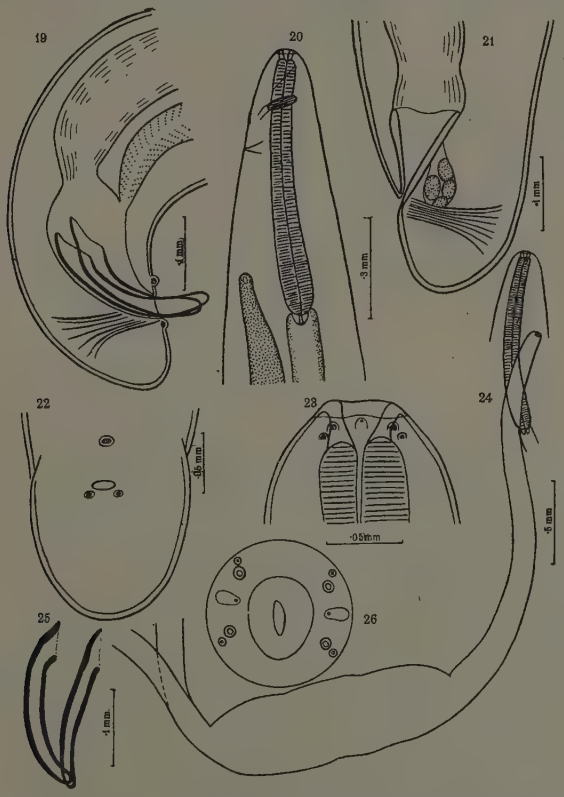
funnel-shaped cavity. The club-shaped oesophagus is not divided into two portions. The oesophago-intestinal valves are typical in structure consisting of three lobes projecting into a fairly thick-walled intestine.

Female: The females measure 17.61–31.42 mm. in length and have a maximum transverse diameter of 0.66 mm. The vestibule is 29μ long and the length of the oesophagus varies from 1.06–1.12 mm. The nerve-ring is .16–.181 mm. and the excretory pore .28 mm. from the anterior end respectively. In all the females examined by the author the rectum is connected to a functional anus. The short and rounded tail measures 118–133μ in length. The vulva is almost flush with the body surface and it is about .5 mm. from the anterior end. There is a long vagina running posteriorly into a sac-like reservoir which gives rise to paired uteri running parallel, posteriorly. One of the uterine tubes, however, sends a loop extending anteriorly a short distance in front of the vulva. The ovaries lie in the posterior portion of the body. The eggs are oblong in shape, measuring 50–59μ long by 28–36μ wide. They contain coiled embryos.

Male: The males, which are more slender and considerably smaller than the females, range from 16.4–21.9 mm. in length and from 0.44–0.46 mm. in maximum thickness. The vestibule measures 26μ in length and the oesophagus is .74–.9 mm. long. The nerve-ring and the excretory pore are .14 and .315 mm. respectively from the anterior end. There is a single testis extending anteriorly into the region of the oesophagus. The spicules are similar and subequal in length, measuring 219–244μ and 235–253μ respectively. The tips are broadly rounded while the heads are slightly expanded but imperfectly chitinized on the ventral aspect. The tail is very short and rounded ; it is 84–114μ long. The caudal alae are lacking but the caudal papillae are well developed and easily discernible. A single median papilla is located in front of the cloaca, while a pair is situated immediately posterior to it.

Discussion: This worm belongs to the subfamily Tetracheilonematinae Wehr, 1935, as defined by Skrjabin and Schikhobalova, 1936. Von Linstow (1901) briefly described the male and later Skrjabin (1917) gave an account of the female and figured its anterior and posterior extremities. Schmerling (1925) erected the genus *Squamofilaria* and designated as type, Rudolphi's "*Filaria*" *coronata*. He defined the genus and re-described the male worm. Since a detailed account of this interesting parasite is not available in the literature, the writer took the opportunity of giving a full description with some new illustrations. The important points to be noted regarding its

morphology are as follows. Previous authors (Skrjabin, 1917 and Schmerling, 1925) describe and figure 6 lip-like festoons surrounding the mouth. Having examined the lateral and end-on views of the head, the writer finds that there are no such structures surrounding the mouth. However, in some specimens, in which the collar is partially contracted, the cuticular elevations corresponding to the cephalic papillae seem to project forwards and simulate the appearance of lip-



Squamofilaria coronata

Fig. 19. Posterior end, male, lateral view. *Fig.* 20. Anterior end, male, lateral view. *Fig.* 21. Posterior end, female, lateral view. *Fig.* 22. Posterior end, male, ventral view. *Fig.* 23. Head, lateral view. *Fig.* 24. Anterior end, female, showing terminal part of reproductive organs with the origin of uteri. *Fig.* 25. Spicules. *Fig.* 26. End-on view of head.

like festoons referred to in earlier descriptions. Yorke and Maplestone (1926) have failed to observe the festoons in *S. pillersi* (Yorke and Maplestone, 1926) and mention only the cuticular collar surrounding the mouth.

As regards the caudal papillae in the male, Schmerling (1925) describes an unpaired median preanal papilla and a postanal papilla. The writer observed that while there is only a single median papilla in front of the cloaca, there are 2 papillae immediately behind it.

A characteristic feature of the spicules in the male is that the heads, while slightly expanded, are imperfectly chitinized on the ventral aspect.

The measurements of the body agree closely with those given by previous authors and call for no special mention. The species has been recorded so far from *Coracias garrula* in Europe. It is here described from a new host in India.

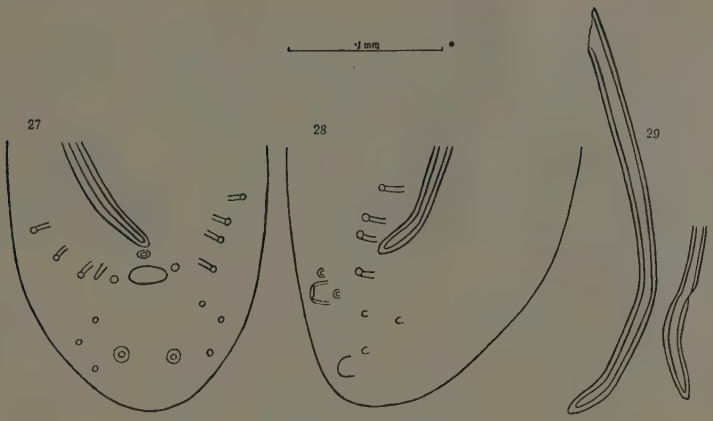
Host: *Coracias benghalensis.*
Habitat: Subcutaneous connective tissue.
Locality: Hyderabad Deccan (The Nizam's Dominions, India).

Subfamily: *Diplotriaeninae* Skrjabin, 1916.
Genus: Diplotriaena Railliet and Henry, 1909.
Diplotriaena tricuspis (Fedchenko, 1874) Seurat, 1915.

This species was recovered on several occasions from the bay-backed shrike, *Lanius vittatus.* Altogether one male and 13 females were collected. This species has a world wide distribution and in India it has been recorded previously from *Trochalopteron jerdoni meridionale* and *Acridotheres ginginianus.* It is here reported from a new host, *Lanius vittatus,* in Hyderabad State.

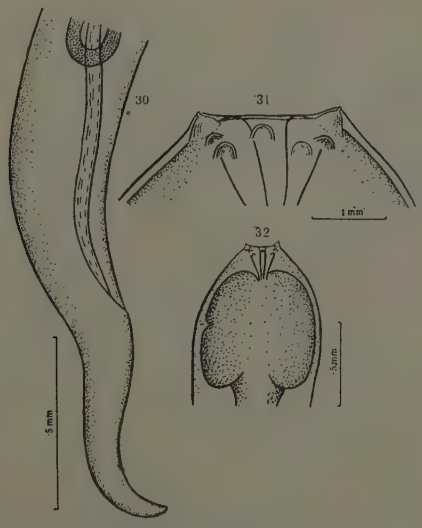
Female: The females vary in length from 45–77 mm. and have a maximum thickness of .66–.9 mm. The tridents are about 240μ long. The nerve-ring is .26 mm. from the anterior end and the excretory pore opens on the ventral surface at about the same level. The total length of the oesophagus is 8.5 mm. and of this .4 mm. is occupied by its anterior muscular portion. The distance from the vulva to the anterior end is .6–1.1 mm. The vagina is a thick walled tube 3 mm. long running posteriorly from the vulva to the paired uteri. The eggs in the vagina and the uteri measure 45–50μ long by 27–31μ wide and contain coiled embryos.

Male: The single male specimen is 24 mm. long and has a maximum transverse diameter of .76 mm. The nerve-ring is .18 mm. from the



Diplotriaena tricuspis

Fig. 27. Posterior end, male, ventral view. *Fig.* 28. Posterior end, male, lateral view. *Fig.* 29. Right spicule and tip of the left spicule.



Avioserpens multipapillosa n.sp.

Fig. 30. Posterior end, female, lateral view. *Fig.* 31. Head, lateral view. *Fig.* 32. Anterior end, female, lateral view.

anterior end. The tail is short, bluntly rounded and without caudal alae. The cloaca is located on a prominence, 85μ from the tip of the tail. There are 4 pairs of pedunculated preanal papillae converging towards the cloaca. A median pre-cloacal papilla and a pair of small sessile papillae are situated in close proximity with the cloacal aperture. Postanal papillae comprise 3 pairs of small sessile papillae with a v-shaped arrangement. In addition to these there is a pair of voluminous papillae placed between the last pair of small sessile papillae. The spicules are unequal and dissimilar measuring 2.928 and .287 mm. respectively.

Host: *Lanius vittatus.*

Habitat: Body cavity.

Locality: Hyderabad Deccan (The Nizam's Dominions, India).

Family: DRACUNCULIDAE Leiper, 1912.

Subfamily: *AVIOSERPENSINAE* Wehr and Chitwood, 1934.

Genus: AVIOSERPENS Wehr and Chitwood, 1934.

Avioserpens multipapillosa n.sp.

A single female specimen of this species was collected once from the pond heron, *Ardeola grayii*. The worm was found in the neck of the bird, alongside the trachea. It measured 105 mm. in length and .72 mm. in maximum width. The body is of almost uniform diameter with a slightly swollen anterior end and a conical tail.

The presence of a glandular oesophageal swelling gives a dome-shaped appearance to the head. It is characterized by the presence of 7 pairs of conspicuous head papillae. Of these, 2 pairs are submedian, one pair lateral and further back there are 2 additional papillae on each side between the laterals and the submedians. The oesophagus is differentiated into several parts. A short anterior muscular portion measuring .186 mm. is followed by a glandular swelling 688μ long by 658μ wide. Posterior to this enlargement the oesophagus is narrowed for a short distance and then expands to form a long posterior glandular part communicating with the intestine. On account of the thickness of the cuticle the junction between the oesophagus and the intestine could not be observed. The latter is a thin tube terminating posteriorly in a short rectum and a non-functional anal opening. The tail is .68 mm. long and constitutes 1/154 of the body length. It tapers gradually to form a pointed tip flexed ventrally. The worm under observation is immature since no fertilised eggs could be found in the genital tubes. The ovaries are long filamentous bodies situated at the

opposite ends of the body and the inconspicuous vulva is 12.25 mm. from the anterior end, dividing the body roughly in the ratio of 1 : 7.5.

Discussion: The genus *Avioserpens* was created by Wehr and Chitwood in 1934 for a worm which they described from a white heron, *Casmerodius albus* or *Egretta thula thula*. The same species was again recorded by Wehr (1934) from the Florida duck, *Anas fulvigula*. In the absence of a detailed description and figures of the type species, it is not possible to compare it in detail with the Indian species. Besides the males of both species have not yet been found. However, the present species could easily be distinguished from *A. denticulophasma* by its smaller size and ventrally flexed tip of the tail. It is therefore concluded that the worm under discussion is a new species and it is proposed to name it *Avioserpens multipapillosa*.

Host: Ardeola grayii.

Habitat: Underneath the skin of the neck.

Locality: Hyderabad Deccan (The Nizam's Dominions, India).

The type specimen will be deposited in the museum of the Department of Parasitology, London School of Hygiene and Tropical Medicine.

Summary.

1. *Buckleyfilaria buckleyi* n.g., n.sp., is described in detail and the new genus is compared with the related genera of Aproctinae.

2. A new species of *Aproctinae* is described and assigned to the genus *Eufilaria* with a slight modification of the generic definition.

3. *Lerouxinema lerouxi* n.g., n.sp., is described from the heart of *Galloperdix spadicea* and its relationship with other genera is discussed.

4. A new species of *Splendidofilaria* is described and compared with the two other species of the genus.

5. *Squamofilaria coronata* from the Indian Roller is described fully and compared with earlier descriptions of the species.

6. *Diplotriaena tricuspis* is recorded from a new host, *Lanius vittatus*.

7. A new species of the genus *Avioserpens* is described from *Ardeola grayii*.

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Bionomics of *Limnaea truncatula* and the Parthenitae of *Fasciola hepatica* under Drought Conditions.

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I.—Aestivation of the Snail.

The non-operculate pulmonate snail, *Limnaea truncatula*, occurs locally in many parts of the British Isles, living in shallow well aerated waters, in marshes, water meadows and similar places. Ellis (1926) notes that the snail is amphibious in habit and more often out of the water than submerged while Boycott (1936) says that it is only occasionally found living in permanent water. The amphibious habit of *L. truncatula* was noted as early as 1883 by Thomas (a) who said that he kept some snails alive for eleven weeks on moist grass and moss. Laboratory observations at Weybridge, where large breeding colonies of the snail have been maintained in connection with experimental work on *Fasciola hepatica*, have amply confirmed the amphibious nature of *L. truncatula* which although able to survive for six weeks or more when submerged usually leaves the water for a mud-bank or the mud-water junction. Our field observations have shown moreover that *L. truncatula* is essentially an inhabitant of temporary ponds and of wet and muddy places which often become dry for part of the year.

Occupation of such a habitat with its alternation of wet and dry conditions means recurrent danger from desiccation to an animal such as *Limnaea truncatula* which depends upon the presence of water for freedom of movement, growth and reproduction. It is of interest to consider the means which the snail adopts to tide over the conditions of drought so characteristic of its typical environment. Since the snail does not appear to have any special devices, such as an operculum, to enable it to resist desiccation it might be assumed that drought in the habitat would lead to the death of all snails, the return of water being followed by recolonisation of the site from adjacent and more favourably situated areas. Recolonisation by flood water or mechanical dispersal have been the usual explanations for the reappearance of *L. truncatula* in habitats which have dried out and remained dry for several months. Thus Thomas (1883 b) described how the waters of the Isis " brought it down in vast multitudes probably from its breeding haunts in marshy places far up the river." to recolonise ditches from which the snail had apparently disappeared. While Thomas's careful observation gives a vivid picture of the way in which *L. truncatula* may

recolonise a habitat, it is not certain that his theory of the means by which it was accomplished is correct.

Permanent occupation of the habitat through a resistant phase in the life-cycle of the snail offers another explanation of this aspect of its life-history and there is evidence from Cheatum (1934) who in America found that seven species of lake pulmonates could survive drought conditions for as long as 62 days mainly through burrowing in wet mud or débris. Thomas (1883 b) did in fact mention dormancy in *L. truncatula* for he found that some snails could survive six weeks' drought in the laboratory and Mehl (1932) said that the snail could resist desiccation for up to four and a half months. Peters (1938) who found *L. truncatula* in habitats which dried out every summer thought that the snails might be able to survive drought for three to four months. All authors agree that *L. truncatula* is notable for its ability to reappear very quickly in habitats from which it has been excluded for long periods by drought, but Walton (1918) was unable to show experimentally that snails buried beneath 2½ inches of damp soil could live for as long as three weeks, which would be insufficient to tide over periods of drought and said " Direct observation in the field and laboratory experiments have both amply proved that the snail can resist drought for a relatively short time." He was of the opinion that the snail's eggs were likely to be the resistant phase although he was puzzled by the appearance on several occasions of one or two large snails in a fresh stock of newly hatched snails following a drought.

Laboratory Observations.

At Weybridge it has been shown that *Limnaea truncatula* is capable of surviving for very considerable periods in the absence of water and that this resistance is of itself quite sufficient to account for the reappearance of the snail in habitats which have remained dry for periods of a year or more. Eggs, juveniles and adults are all resistant, the eggs being apparently less resistant than the snails themselves. Even newly hatched juveniles which might be expected to be particularly susceptible to starvation while aestivating, are resistant for considerable periods.

Aestivation of Newly Hatched Snails.

In the laboratory, adult *L. truncatula* in small covered culture dishes with mud slopes and a small pool of water, deposited egg masses which hatched in 12–13 days at air temperatures of about 60°F. to 70° F. Immediately hatching was noted the covers of the culture dishes were removed and the contents allowed to dry by natural evaporation in a

well lighted laboratory where air temperatures ranged from 55° F. to 70° F. The mud retracted from the sides of the dish, becoming superficially quite hard and dry four days later, when the newly hatched snails were observed in various places on the mud with the shell aperture closely applied. The effect of drought conditions on the aestivating snails was assessed by removing snails at intervals and noting their rapid recovery when placed in water. Snails were successively removed from one dish over a period of 60 days and all were found to become active when placed in water. After sixty days no more snails were apparent in the dish which was accordingly flooded with water, when four more snails soon emerged and commenced to feed. Growth thereafter was extremely rapid and maturity was reached within three weeks. There was no evidence of any mortality in the snails of this group, but snails in a second culture dish exposed under similar conditions were all dead when the dish was flooded with water 78 days after the mud became dry.

This indicated that newly hatched snails, which can have had little if any opportunity of feeding in the interval between hatching and the onset of drought conditions, were able to survive at least two months' exposure on a hard mud surface in the dry atmospheric conditions of the laboratory. It seemed probable that this did not represent the maximum period of viability for *L. truncatula* under such conditions and in fact larger snails were observed to remain alive in aestivation for considerably longer periods.

Aestivation of Older Snails.

The observation was carried out in the laboratory using half-grown (0.30 cms. to 0.50 cms. in length) laboratory reared snails kept in small glass culture dishes on mud slopes with a little water which was allowed to dry out slowly, as described above, by natural evaporation in the laboratory. A few snails seen in the dishes with apertures exposed were found to be dead after four days when the mud looked dry and had started to crack. Most snails, however, survived for much longer periods, all ten snails of one group being alive after six months. Several groups which commenced aestivation in December, 1947, were examined at intervals to assess the viability of the snails and the last to be examined was flooded with water on the 14th December of the following year when three snails, out of ten originally placed in the dish, were found to be alive. This suggested that under suitable conditions a high percentage of aestivating snails might survive for a year in a dry habitat.

There is no reason to regard this as the limit of endurance although it is in fact the longest period so far recorded in the laboratory. Under different experimental conditions other groups of snails were viable for shorter periods ; for example 45 snails were induced to aestivate on mud slopes in small unglazed earthenware pans but of these only eight survived when the pans were flooded with water six and a half months later. Evidently the earthenware pans allowed greater desiccation and were not as suitable for long term aestivation as were glass culture dishes which retained a higher degree of humidity. Some degree of variation in resistance according to local conditions may be expected and it is thought that the laboratory results are far behind the maximum possible in the field.

Factors influencing Snail Survival under Drought Conditions.

It is evident that the conditions under which aestivation occurs will have a marked effect on the survival rate of snails and many different factors influence the chance of survival of each individual. Walton (1918) concluded that the time necessary to cause death depended upon physical and meteorological factors which were very variable while Mehl (1932) stated that the degree of survival depended on such factors as temperature, soil type, available shade, depth to which the snails entered the soil and closure of the shell orifice. Thomas (1883 b) called attention to the importance of grass in preserving the humidity of the earth where the snail was living and Peters (1938) described finding living *Limnaea truncatula* beneath the moist layers of an algal mat on the bed of a stream. Observation has suggested that it is particularly necessary for the snail to enter into aestivation with the shell aperture closely applied to the mud surface on which it is living. At the laboratory and in the field snails are often found closely applied to the mud and in addition with a considerable plug of mud which occludes the shell and reduces the rate at which dehydration occurs. Contrary to an observation by Rees (1932) the presence of an epiphragm in *L. truncatula* has not been observed and this suggests one reason why snails which start aestivation with the shell aperture exposed seem to die within a few days. Walton (1918) first mentioned that the snail shows little inclination to follow receding water and observation at Weybridge has suggested that the rapid onset of drought will leave many snails exposed to extreme desiccation which appears to prove fatal. Under circumstances where the habitat dries out more slowly the habit of the snail of feeding at the mud-water junction will tend to ensure that many snails reach the lower and hence the permanently

moist parts of the habitat before all the surface water disappears. Actual burrowing into soft mud, as described by Olsen (1944) for *Stagnicola bulimoides techella* has been observed with *L. truncatula* at Weybridge but in the field it is probable that retreat of the snail into cracks in the drying mud or accidental covering from the trampling of stock are of more importance. Field observation has shown *L. truncatula* in aestivation beneath the superficial crust of mud covering the shallow mud-pans which had served as its breeding place in the wet months of the year, while earth and grass samples taken from dried-out habitats will often be found to contain viable snails which will emerge when the sample is covered with water and left to soak for twenty-four hours. In this way the existence of *L. truncatula* may be demonstrated in localities where it has not been found by careful field observations.

Breeding of *Limnaea truncatula* following Aestivation.

In the field the periods of drought which the snail normally has to endure are much shorter than the possible period of viability which has been suggested from experimental evidence. Characteristically, *L. truncatula* is found in places subject to flooding or where the rainfall is high. Walton (1918) who investigated *L. truncatula* in the Aberystwyth area, mentions that the longest dry spell between 1901–1915 was of 23 days and that on the average dry periods of more than ten days were rare while all winters were sufficiently wet for *L. truncatula* to reach breeding size by spring. Hence because *L. truncatula* has such powers of withstanding intermittent dry periods in its habitat it will be likely to survive in numbers under the usual climatic conditions of Britain even in areas that frequently appear dry.

Laboratory Observations.

In the laboratory, young snails with adequate food supplies grow with astonishing rapidity and at air temperatures of 60° F. to 70° F. a proportion attain a shell length of 0.5 cms. and commence laying 21 days after hatching. Thereafter for several weeks large numbers of egg masses, each containing up to 25 eggs, will be laid and hatched in 12–13 days with little or no mortality. One snail under observation at the laboratory actually produced 394 eggs in 16 days from the first date of laying thus indicating a potential second generation of 160,000 snails which might appear after about twelve weeks. One snail, placed in a large culture dish, did succeed in giving rise to about 25,000 in this period of time.

Since the snail is self-fertile, the minimum survival requirement is one individual out of a whole colony, the extremely rapid rate of

reproduction making adequate compensation for mortality occurring during a drought. Periodic dryness in the habitat may actually increase the supplies of food available to the snail for it seems that *L. truncatula*, which feeds largely on unicellular algae such as the Desmid *Cosmarium*, is particularly suited by the ecological conditions of a mud bank alternately subjected to flooding and drought, exposed and denuded by trampling, dried hard in the sun and then, after rainfall or surface water encroachment, rapidly covered with a green film of algal growth which is avidly eaten by the snails.

Conclusion.

It is shown that *L. truncatula* is very resistant to the conditions of drought which are so characteristic of its typical habitat. Drought in the environment leads to aestivation of juvenile and adult snails while the return of water to the dried habitat is likely to be followed by very rapid recolonisation from the multiplication of the progeny of even a few snails which may have survived.

II.—Bionomics of *Fasciola hepatica* in Aestivating Snails.

The resistance of *L. truncatula* to drought is of obvious importance in considering the epidemiology of the disease of fascioliasis in domestic stock in Britain. Since it appears that the snail is able to survive for a year or more in aestivation under drought conditions it is of importance to discover whether snails infected with *F. hepatica* are equally resistant and whether the parasite remains alive in its aestivating host. Olsen, who in 1944 described the life-history of *Stagnicola bulimoides techella* (the Texas vector of *Fasciola hepatica*) and showed that young *S. bulimoides* could aestivate in the soil for as long as five months, further reported in 1947 that infection with *Fasciola* survived the summer drought. It will be shown similarly, in the present paper, that *F. hepatica* can survive the dormant periods of its host *L. truncatula*. There is evidence, moreover, that the parasite continues developing in the snail during the period of aestivation and is therefore to a great extent protected against the climatic hazards of the environment. This offers an explanation of the difficulty described by Peters (1938) of understanding the way in which *Fasciola hepatica* manages to complete its life cycle in such an " elusive intermediary " as *Limnaea truncatula*.

Laboratory Observations.

At Weybridge interest in the possibility of the development of *F. hepatica* in aestivating snails was aroused by evidence of the survival of another trematode species in *L. truncatula* which was known to

have been dormant for three months. In June of 1947 examination of *L. truncatula* from a district in Yorkshire showed that nearly all the snails were infected with an unidentified stilate cercaria. Snails from this collection were placed in water in a large earthernware dish which was allowed to become dry by natural evaporation. Three months later, when thirty snails removed from the dry dish were placed in water, it was found that sixteen had survived the considerable degree of desiccation and within a few hours became active. Within 48 hours stilate cercariae emerged from twelve of these snails thus indicating that a trematode parasite was able to survive the aestivation of *L. truncatula*. It seemed that *F. hepatica* might behave in a similar way and laboratory experiments were devised to investigate the possibility.

Bionomics of Recent Infections in Aestivating Snails.

To determine whether *Fasciola* could survive the dry-period dormancy of the snail an experiment was arranged using 170 snails (measuring 0.25 c.ms. to 0.47 cms. in length) which were each exposed to infection with ten miracidia, experience having shown that this number is sufficient to ensure infection in all snails. After 24 hours' exposure to infection the snails were placed in a laboratory where they were kept under good environmental conditions at air temperatures which ranged from 50° F. to 70° F. Dissection after three weeks showed that the majority of the snails contained well developed sporocysts of *Fasciola* while a few contained first stage rediae. Groups of ten snails were then moved to small culture dishes each of which held a slope of sterilised mud which had been inoculated with algae suitable as food for the snails. Thereafter some groups were kept under moist conditions by the addition when necessary of filtered pond water while others were allowed to become dry by natural evaporation in the laboratory. Four days later the exposed mud slopes were dry and the snails had become quiescent. The two series were maintained at laboratory temperatures, the one under moist and the other under dry conditions, for the rest of the experimental period. On February 18th, 1948, about three months after infection, cercariae of *Fasciola hepatica* first began to emerge from snails in those groups which had been kept under moist conditions where the snails led a normal active existence and by the end of the month all of the snails were shedding cercariae. Snails from some of the dormant groups in the dry dishes were examined at this time to ascertain whether their parasites were still alive and if so, how far development had proceeded. Examination of one group of ten snails which had remained dormant for three

months showed that nine soon became active when placed in water and while no cercariae emerged from these snails during the next 48 hours, dissection showed that the parasites were still viable and that development had proceeded to the extent that a limited number of cercariae had matured. One snail measuring 0.43 cms. in length contained numerous rediae of which one had developed a fully mature cercaria while there was a free and active cercaria in the body cavity of the snail.

Observation of the groups in aestivation was continued throughout 1948 until the last was examined in October. Of ten snails originally in this group, six were found to be dead, while the others all contained rediae and a few active cercariae. It appears therefore that *F. hepatica* can survive at least ten months dry-weather dormancy on the part of its snail host and it seems probable, in fact, that under such conditions the parasite will survive until the death of its host. As described earlier in this paper, uninfected snails are able to survive for at least a year in aestivation and there is every reason to believe that this period might be extended under more favourable conditions.

While infections survived an extended period of dormancy it was interesting to note that the stage of development of the trematode was less advanced than in those snails which had led an active existence. All infected snails leading an active life developed within seven or eight months 150 to 300 rediae which were shown on dissection to be packed with mature cercariae and well developed embryos while in addition the body cavity of the snail contained up to 1,000 active cercariae. On the other hand, snails which had been in aestivation developed very few mature cercariae and the rediae found on dissection were few in number, small in size and contained undifferentiated germ buds. Aestivation of the snail obviously retarded development of the parasite to a marked degree, the effect appearing more marked with extension of the dormant period. After ten months in aestivation the largest number of cercariae found in a snail was fifteen, and in one snail there were no cercariae at all and only two rediae. Moreover, as will be shown below, aestivation of the snail in addition to retarding the development of a young infection appeared also to reduce the number of cercariae and rediae in an infection which was mature at the onset of the dormant period.

Bionomics of Mature Infections in Aestivating Snails.

The effect of aestivation of the host in reducing the numbers of established rediae and mature cercariae was well illustrated in 100 snails which were known to contain large numbers of mature rediae

and cercariae and which were induced to aestivate in February, 1948. At this time dissection of snails from the group showed the presence of an average of about 160 rediae while between 50 and 300 cercariae emerged from each snail during 24 hours' observation of a random sample. By July dissection of a sample of the aestivating snails showed that very numerous rediae were still present but that none seemed to contain mature cercariae while very few free cercariae remained in the body cavity. Snails which had been kept under good environmental conditions were found to contain as many as 700 mature cercariae and nearly all the rediae contained cercariae in various stages of development. One live snail was recovered in November, 1948, when the dish containing the aestivating group was flooded with water and on dissection it was found to contain only six mature cercariae with a number of immature rediae. This observation suggests not only that the development of cercariae is retarded during aestivation but that large numbers of mature cercariae disappear ; presumably they die and are absorbed.

Bionomics of Fasciola *in Snails following Aestivation.*

It has been shown earlier in this paper that *L. truncatula* grows to maturity very quickly on the return of moist conditions to its habitat after a prolonged period of drought. In the same way, *F. hepatica*, the growth of which has been retarded by aestivation, will undergo extremely rapid development when its host once again becomes active. Dissection of a group of snails which had been kept in aestivation for approximately four months showed that there were very few mature cercariae and that most of the rediae contained undifferentiated germ buds. Snails which were brought back to activity after being placed in water grew rapidly under good environmental conditions and their contained parasites developed similarly. After six weeks as many as 300 cercariae were observed to emerge from a single snail during a period of 24 hours. On the other hand there had been no such rapid development of the parasites in snails which had remained in aestivation, these containing a few ill-developed parasites as before.

It seemed therefore that there was a relationship between the growth of the snail and the growth of the parasite and this was demonstrated particularly well with a group of snails which had remained in aestivation for a period of six months. After activation in water half of the living snails in this group were placed in dishes under good environmental conditions and with plenty of available food. They made rapid growth and dissection carried out at the end of seven days showed the presence of very many large rediae, each of which contained two

or three apparently mature cercariae as well as many developing embryos, while during an observation period of 24 hours a total of 258 cercariae emerged from four snails of the group.

No cercariae emerged, however, from a similar number of snails which after aestivation had been kept in filtered pond water without access to food. Dissection of one of these snails showed the presence of 56 rediae measuring not more than 650μ in length and containing relatively undifferentiated embryos with a very few mature cercariae. Failure of the parasite to develop after a period of aestivation thus seemed to be related to the inability of the host to obtain sufficient food for normal growth.

Relevance of the Observations to the Epidemiology of Fascioliasis.

These observations suggest new and interesting aspects of the disease caused by *F. hepatica* in domestic animals. One of the difficulties in field control has been the demonstration of the presence of the snail which often appears to be absent from areas where liver-fluke disease is known to be endemic. It is now evident that apparently unsuitable habitats may harbour the snail which can multiply exceedingly rapidly when the environment changes. Infection with *F. hepatica* survives dry-weather dormancy of the snail and the parasite develops rapidly to maturity as the snail grows and multiplies under favourable conditions. These aspects of the life-history of the snail and its parasite explain at least in part the occurrence of outbreaks of the disease of fascioliasis after its absence from particular areas for many years.

Discussion.

It has been shown that *F. hepatica* can survive for ten months while its host *L. truncatula* is in a dormant state and since there is no evidence that snails lose an infection once acquired there is little doubt that the parasite would survive for longer periods in snails aestivating under exceptionally favourable conditions. Although, however, *F. hepatica* could be demonstrated in snails which had survived a prolonged period of dormancy, development of the parasite was severely retarded as a result of the inactive state of the host and after a time very few of the most mature parthenitae (the cercariae) could be found. After the termination of the dormant period rapid development of the parasite occurred only if the snail host had access to adequate supplies of food. This suggested that food was an important factor in controlling the rate of development of the parasite in the aestivating snail. As will be shown in a subsequent paper, the rate of development of *Fasciola*

in active snails is closely related to their plane of nutrition, for parasites in active snails which are kept under starvation conditions develop very slowly. There is a close analogy between such snails and those which are aestivating, for in both instances they are deprived of food. The conclusion that food is an important factor in determining the rate of development of the parasite in its dormant host received support when the examination of successive groups of dormant snails showed that the slow rate of development of the parasite was less apparent during the earlier than during the later stages of aestivation, an understandable circumstance since the digestive gland contains much more food at the beginning than later in the period of aestivation.

It is necessary to conclude that the development of the parasite is adjusted to conserve the reserves of the host. During aestivation, which appears characteristic of the life-history of *L. truncatula* excessive demands made by the fluke would result in the premature death of the host and hence of the parasite. In fact, although the numbers available for comparison were not very large, it did not appear that aestivation increased the differential mortality usually observed between groups of infected and groups of uninfected snails. This may be ascribed to the high degree of adaptation which has developed between the two species *Limnaea truncatula* and *Fasciola hepatica*.

Summary.

1. Field and laboratory observations confirm that *L. truncatula* is an amphibious species, living in temporary pools and muddy places which often become dry for part of the year.

2. During times of drought the snail survives in a state of aestivation. At the laboratory newly hatched snails are able to withstand at least two months' aestivation while older snails have been demonstrated to remain alive for more than a year; considerably longer than suggested by previous workers.

3. *Fasciola hepatica* in its snail host survives drought for comparable periods and rediae and cercariae have been demonstrated in snails known to have been dormant for as long as ten months.

4. An important new aspect of the host-parasite relationship arises from observations showing that *F. hepatica* not only survives but undergoes development in aestivating *L. truncatula*.

5. During aestivation development of the parasite proceeds slowly, apparently restricted by the limited amount of food available from the dormant host but at the end of the drought period and under good environmental conditions, both host and parasite develop rapidly.

6. It is suggested that these new aspects of the ecology of *L. truncatula* and of its relationships with *F. hepatica* are likely to prove of importance in the control of fascioliasis among domestic animals.

ACKNOWLEDGMENTS.

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On *Capillaria cadovulvata*, pathogenic to *Perdix perdix*.

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Capillaria cadovulvata was first described in 1945 by Madsen who found that it occurred commonly in the caeca of adult pheasants, and occasionally among partridges in Denmark. It is recorded now from a grey partridge *Perdix perdix* in England, where it had set up pathological changes which had been the cause of death. Madsen does not mention the presence of any lesions in association with this parasite. Though this is the first record of the species in England, it may be more common, for the species of *Capillaria* are not always easy to differentiate without recourse to fairly high magnification. In view of this, and because of its pathogenic nature, it seems worth while recording its occurrence and the lesions that may follow its presence.

The bird concerned was an adult hen partridge (*Perdix perdix*), which had been hatched and reared on the Game research estate of Imperial Chemical Industries Ltd. in Hampshire, and was reported as being in poor condition before its death on 5th February, 1949. It was emaciated, weighing only 180 gm.; the feathers were lustreless and there was some muscular inco-ordination though actual paralysis was absent. The eye was dull and movements were slow and erratic. Appetite was not apparently impaired, and after death the crop and gizzard contained a reasonable quantity of food.

At autopsy lesions were seen only in the left caecum. This was distended, thickened and pale. A smear of the contents revealed the presence of large numbers of ova of a species of *Capillaria*. On opening up the caecum under water however, only two worms floated out from the scanty contents. In the other caecum there were four worms, all of which were diagnosed as *C. cadovulvata*, on the basis of size, structure of the genitalia and the presence of a post-vulvar swelling in the females, though the glandular structure described by Madsen on this swelling was not obvious.

The diseased caecum had few contents, consisting of a little faecal matter and a quantity of flocculent mucus. After washing out these contents there could be seen a mild generalized typhlitis and two areas which showed considerable change. One area, near the fundus, was roughly circular in outline and measured about 2 cm. diameter. It was anaemic and appeared to be covered with a pseudomembrane over a pulpy core. The other area was smaller, rather pale and soft.

From sections it was possible to work out the course of the disease. The worms, both male and female, thread themselves into the tissue of

the caecum and the whole depth of the serous coat is involved. The muscular layers are not invaded, though the lymphatics here are slightly distended and there are a few leucocytes. The whole of the oesophageal region of the worm is embedded, and in some cases part of the intestinal section as well, so that some ova are actually deposited within the host tissue, though many are deposited directly into the lumen. The immediate host reaction is typically inflammatory with a deposition of leucocytes, mainly eosinophiles and lymphocytes, round the invading body. There is general hyperaemia and fibrocytes appear in an attempt to wall off the worm with a connective tissue cyst. There is an extensive cellular infiltration throughout the whole of the serous layer, even where no ovum or worms can be demonstrated. This stage is followed by marked necrosis and sloughing of the epithelium. The villi and glandular structures become completely disorganized, forming a gangrenous area of broken down decomposing cells, haematin from extruded blood, serum and a mass of leucocytes. This eventually becomes covered with a pseudomembrane formed of serum, fibrin and leucocytes. In the course of this destruction many ova must be released from the tissues to pass out with the faeces, though others can be seen still caught up among the glandular tissues of the caecum, each with its own individual inflammatory reaction. Haemorrhage is only slight but there is much congestion and engorgement of the blood vessels including the lymphatics. There was no evidence of serious secondary bacterial invasion, though such an occurrence is not unlikely as a sequela to helminth lesions.

The life history of this parasite is being worked out, for it may prove to be a factor of some importance among phasianid birds. Various species of *Capillaria* are known to be pathogenic, notably *C. annulata* and *C. contorta* which are found in the upper digestive tract, while the present writer has recorded intestinal lesions and rupture caused by the presence of *C. longicollis* among partridges. Until we have much more experience we cannot say if *C. cadovulvata* is normally pathogenic or if there was a prior lesion in the caecum to which the worms were attracted, such as happens when *Enterobius vermicularis* invades a diseased appendix. But it is obvious that it is a potential pathogen and as such must concern all who are interested in the health of game birds—and perhaps poultry. I am indebted to Mr. R. J. Fant who took the photographs illustrating this article.

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Capillaria caudinflata

1. Egg in caecal mucosa. 2. Longitudinal section of adult worm embedded

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A Case of Human Gnathostomiasis in Malaya.

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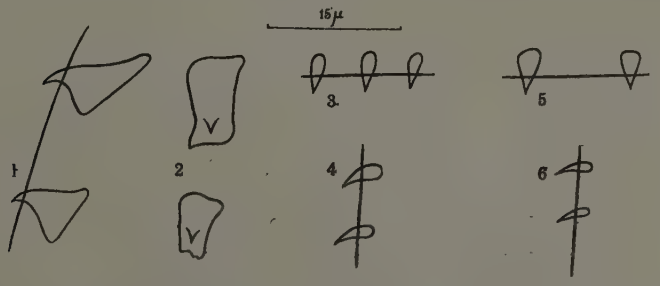
Twenty-eight cases of adult or larval worms belonging to the genus *Gnathostoma* Owen, 1836, have been recorded from man.

One of the earliest records is from Malaya where Samy (1918) extracted a larval gnathostome from the finger of a young male Chinese.

In the present case (the second record of human gnathostomiasis from Malaya) the worm was removed surgically on 19th November, 1947, from the pulp of the right middle finger at the advancing end of a raised linear track (1.0 mm. wide) which extended from a septic swelling over the nail bed. The skin in front of the track was healthy.



Gnathostoma larva from Malaya.

Fig. 1. Cephalic spines, side view. Fig. 2. Cephalic spines, front view. Fig. 3. Body spines, of anterior portion, front view. Fig. 4. Body spines, of anterior portion, side view. Fig. 5. Body spines, at ¼ length of body from collar, front view. Fig. 6. Body spines, at ¼ length of body from collar, side view.

The worm appeared as a black object under the skin and had taken five days to travel from the dorsum of the finger to the pulp. Removal of the worm was followed by rapid subsidence of inflamatory changes on the finger and there had been no recurrence of the complaint when the patient was interviewed two months later.

The patient was a Chinese woman (Cantonese) of 36 years of age who had emigrated from South China at the age of seven and had lived since at Menglembu, a village near Ipoh in the State of Perak (Malaya).

The worm, which had been in normal saline for 9 days before examination, proved to be a larval gnathostome. The mouth is provided with two large fleshy lips behind which is a cephalic bulb with four rows of backwardly directed simple spines. The spines on the cephalic bulb are about 10 to 13μ long and have broad roots. Four cervical sacs are visible at the anterior end of the worm. Genitalia are not developed. The anus is sub-terminal with the tail ending in a blunt point. Total length 3.0 mm., maximum width (at about middle of body) 0.6 mm., diameter of cephalic blub 0.35 mm., length of cephalic bulb 0.25 mm. There are twenty-four rows (extending to slightly more than one-third of the length of the body) of simple spines at the anterior portion of the worm. These spines are not leaf-like and have no denticulations. The cuticle of the posterior part of the worm is smooth.

Clinical experience of Prommas and Daengsvang (1934) and Castens (1935) suggests that human gnathostomiasis is probably commoner in Siam than is generally believed. *Gnathostoma spinigerum* Owen, 1836 and *G. hispidum* Fedtschenko, 1872, have been recorded from domestic animals in Malaya. A section of emigrant Chinese indulge in eating raw fresh-water fish in Malaya and human gnathostomiasis may be not uncommon in this community, but hitherto has escaped notice.

It is probable that the specimen is a larval *G. spinigerum* but in the absence of a more intimate knowledge of variations in cuticular spines in the developing forms of gnathostomes, it is considered unsafe to attempt species determination especially on larval forms obtained from abnormal situations in accidental hosts. The spines show some of the peculiarities discussed by Mukerji and Bhaduri (1945) in that those on the cephalic bulb are simple but have broad roots of varying shapes and those on the body are not flattened with denticulations, but simple.

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Potato Root Eelworm, D-D, and Soil Sterilization. III. Results for 1947.

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In two previous papers the writer has described a factorial experiment to test the effects, on potato plants growing in pots of soil, of the three factors: (S) steam-sterilization of soil, (H) infection of soil with about three million eggs and larvae of *Heterodera rostochiensis* per pot, and (D) injection of each pot with 10 ml. of D-D, the treatments being applied in that order to half the pots so as to yield every possible combination. The first paper dealt with the methods used and with the selection of two criteria, out of ten tested, which brought out the significant effects. The second paper discussed in detail the results obtained in the first season (1946); these included (i) no definite evidence of any eelworm effect, (ii) a very marked positive effect due to steam sterilization, and (iii) a less marked positive effect from D-D even in the absence of infection.

These results appeared to justify a repetition of the experiment in 1947 in the sense of growing another crop of potatoes in the same pots without renewing the treatments, with the object of seeking residual effects. The forty 13″ pots were stored in an unheated shed during the severe winter of 1946/7 with the result that several of them crumbled and the set could not be used again. The next available size, 8″ high and 10″ in diameter, held less than half the amount of soil held by the former and, no more than fivefold replication being necessary, it was decided to introduce a fourth factor represented by a dose of 4.7 gm. per pot (4 oz. per square yard) of a mixed artificial fertilizer. Each individual pot of soil from the first season was thus replaced by two pots, one with and one without fertilizer. The analysis of the 80 pots then becomes:

Source of Variance	*Degrees of Freedom*
Original treatments	7
Fertilizer	1
Interaction	7
All treatments	15
Error	64
Total	79

Eighty "Duke of York" potatoes of mean weight 62 gm. were planted (31/3/47), and the pots were freely randomized for position in

two rows of 40. Heights of haulms were taken at 45 and 78 days after planting, the number and weight of all tubers was determined after 87 days, and the colour of haulms was assessed at 84 days to test for differential degrees of withering (this criterion yielded nothing). Cyst counts were made from single 200 gm. soil samples, and larval counts (as before) from duplicate aliquots from each of two batches of 100 cysts each. On this occasion unhatched eggs and hatched larvae were separately counted. Logarithmic transformations of cyst and larval counts were again used for analysis.

Plant Criteria.

Of the five criteria tested, it was again found that height at 45 days and weight of all tubers were the most satisfactory, and these have therefore again been selected for detailed analysis. All criteria were considerably more variable in 1947 than in 1946 and this, so far as plant criteria are concerned, is ascribed to the greater variability in the size of the 80 tubers used. Moreover in 1947 plants were much smaller and produced fewer and smaller tubers than in 1946 ; the average weight of all tubers was 16.5 gm. in 1946 and 9.7 gm. in 1947. This reduction is not mainly due to soil impoverishment since even the plants receiving fertilizer were much inferior to those of 1946. It must be ascribed largely to the use of smaller pots and to the fact that the sets were planted about a month earlier.

There were in all 16 treatments with five replicate pots in each ; the 16 treatment means are shown in Table I together with the mean and standard deviation. The contrasts obtained by dividing the 80 pots into two sets of 40, in various ways, yield the mean treatment differences which measure the effects of factors, listed in Table II along with the standard error of the difference.

Analysis of variance, following the scheme noted above, showed that both fertilizer and original treatments gave a mean square significantly greater than error. The interaction between fertilizer and original treatments was significant only in the case of height at 45 days, and here the original treatments mean square was not significantly greater than the interaction mean square. This situation has produced a crop of significant effects in the first numerical column of Table II, including a significant quadruple interaction. Omission of the 40 fertilizer pots gives the slightly less exuberant array of significant effects shown in the second column of the Table. The other criterion, weight of all tubers, is not complicated by a significant interaction between fertilizer and original treatments.

TABLE I.

Summary of Treatments : Means per plant.
(Based on 5 plants per treatment.)

Treatment	Height of Haulms at Day 45 (cm.)	Weight of all Tubers (gm.)
Control	10.2	78.4
(h)	6.4	35.6
(s)	10.4	67.6
(d)	8.6	67.0
(hs)	5.8	27.6
(hd)	9.4	75.2
(sd)	10.8	61.6
(hsd)	11.8	76.2
(f)	15.6	100
(hf)	5.8	48.6
(sf)	9.8	119
(df)	11.6	88.8
(hsf)	10.4	35.4
(hdf)	12.8	78.8
(sdf)	10.8	102
(hsdf)	12.8	88.0
Mean :	10.2	71.8
s :	2.48	24.3

TABLE II.

Effect of Factors : Mean differences per Plant (based on 40 plants with, minus 40 without the factor ; except the partial height data, based on 20 minus 20). Values in **bold** type are significant.

Factor	Height of Haulms at Day 45 (cm.)		Weight of all Tubers (gm.)
	(Full)	(Partial)	(Full)
H	**—1.58**	**—1.65**	**—27.3**
S	0.28	1.05	0.6
D	**1.78**	**1.95**	**15.7**
HS	**1.33**	—0.15	—3.3
HD	**2.83**	**2.55**	6.3
SD	0.68	1.25	3.9
HSD	—1.08	0.25	3.9
F	**2.03**	—	**21.4**
HF	0.08	—	**—12.3**
SF	—0.78	—	6.4
DF	—0.18	—	—2.0
HSF	**1.48**	—	—5.6
HDF	0.28	—	0.6
SDF	—0.58	—	0.3
HSDF	**—1.33**	—	3.0
S.E. of Difference	0.554	0.784	5.44

Inspection of Table II, and comparison with the corresponding Table II in the second paper of this series, will at once reveal the main points brought out by this repetition of the experiment. These are as follows :

(i) *Eelworm effect.* There is a significant negative effect under both criteria, and under the second it is the largest effect of all factors in the Table. This is in marked contrast to the 1946 results, where no significant eelworm effects were found.

(ii) *Steam-sterilization effect.* This, by far the largest effect in 1946, is no longer significant, and indeed is slightly negative in the other three criteria.

(iii) *D-D effect.* As in 1946, this effect is still significantly positive.

(iv) *Original interactions.* Except in the complete analysis of height, which is suspect, there is again no significant HS interaction, and there is again (under height only) a significantly positive HD interaction. All sign of the negative SD interaction of 1946 has disappeared.

(v) *Fertilizer effect.* As expected this has been large and positive ; in fact it is the only factor which was significantly positive under all five criteria. It is interesting that, under neither criterion was it the largest effect produced.

(vi) *Fertilizer interactions.* Apart from the HSF and HSDF interactions in the full analysis of height, which are of doubtful meaning, there is a significant negative HF effect under the weight criterion.

As in the case of the 1946 results, it is again considered desirable to analyse the main factors into two components, namely their effects in the presence and in the absence of each of the other factors : this is done in Table III, which is comparable with Table III of the second paper. Each double column relates to the effects of the factor quoted at its head, and each pair of values within the column to its effects in the presence and absence of the second factor quoted to the left ; the algebraic sum of each pair is given in the " Total " line, and one-fortieth of this sum is the " difference per plant " of Table II.

Eelworm Infection : H.

The negative eelworm effect shows interesting features in Table III, and some differential response as between the two criteria. Thus, under height, H is significantly negative only in the absence of any one of the other factors : it would appear that the presence of S, D, or F is sufficient to counteract any harmful influence due to H. Under the weight criterion, it is again true that H is negatively significant only in the absence of D, but with respect to S and F the larger negative

effect occurs only in their presence. One important difference between these two criteria is the time of their determination : respectively 45 and 87 days after planting. If the various factors are components in a changing dynamic equilibrium, with H hostile to plant growth, D favourable to it but hostile to eelworm, S and F favourable to plant growth and therefore to the growth and multiplication of eelworms, then the situation might be interpreted as follows. D-D has exerted its nematocidal effects during the previous year ; Heterodera has therefore a negative effect only in the absence of D-D, under both criteria. Both steam sterilization and artificials encourage plant growth, and by the 45th day this stimulus is able to outweigh the negative eelworm effect so far as height is concerned. By the 87th day, however, plant stimulation has in turn led to an eelworm infestation sufficiently increased to reduce the size of tubers produced (the reduction in *number* of tubers was not significant). From a consideration of the way in which these eelworm effects are measured, by contrasting one group of treatments with another, it can be seen that the eelworm effect in the absence of D-D involves no bias, since the bias is due to the lethal action of D-D on eelworm. In the presence of D-D, however, the contrast reduces to zero if all eelworms are killed by D-D, and in this case it would be expected that only random differences should occur, as happens in Table III.

When the eelworm effect is analysed in respect of presence or absence of steam sterilization, and of fertilizer, however, both components (presence and absence) share the bias in such a way that the true measure of effect is diluted by about half.

Thus there is no reason to reject any of the significant values in the first numerical column of Table III. The values for " D-D absent " are unbiased ; those for " D-D present " are probably estimates of zero. In the presence and absence of the other two factors the actual values tabulated, and the comparisons between presence and absence, are unbiased but diluted.

Soil Sterilization : S.

So far as steam sterilization is concerned, no criterion gave any significant effect from this factor as a whole. In the face of this situation, the positive effect of S on height in Table III, in the half of the pots which contained eelworm, is of doubtful significance. It might be a reflection of the already noted fact that eelworm fails to show its negative effect on height in the presence of steam-sterilized soil, but it is safer to regard the differences under the heading S in Table III as merely random.

D-D Injection: D.

The significantly positive D-D effect is seen from Table III to occur under both criteria in the presence of eelworm: in its absence the effect is negative though not significantly so. In the previous year the D-D weight effect was significantly positive both in the presence and in the absence of eelworm, and under the latter condition the effect was taken as evidence of a " soil-amendment " action. It would thus seem that the soil amendment effect has entirely disappeared in 1947, in which case the positive effect in the " presence " of eelworm might be interpreted as due to the nematocidal action of D-D in the previous year; the eelworms " present " are, in fact, mainly dead and therefore unable to exert their negative effect. As explained in the previous paper, this leads to a bias in the " Eelworm present " contrast. The full D-D contrast is:

$$D = \{ (d + sd + df + sdf) + (hd + hsd + hdf + hsdf) \} \quad \ldots \quad (A)$$

$$- \{ (O + s + f + sf) + (h + hs + hf + hsf) \} \quad \ldots \quad \ldots \quad (B)$$

The bias occurs in the second half of expression (A) in each term of which H and D occur together and the eelworms are largely killed by the D-D. Thus their negative effect is mainly neutralized and expression (A) becomes more strongly positive than it would otherwise be, irrespective of the D-D effect.

In the third numerical column of Table III, where the effect of D-D is divided into two components: namely, in the presence and absence of the other factors, the contrast " H absent " is, in fact, the first half of expression (A) minus the first half of expression (B). The contrast is free from bias. The contrast " H present " is that of the second halves of the two expressions, and contains maximal bias. If one regards the H factor as eliminated (by D-D) from the first part, and the other factors as cancelling out, the contrast reduces to:

$$D = 4(d) - 4(h),$$

instead of $D = 4(d)$, and one would expect this to be positive even if D-D were without effect, owing to the negative effect of H.

Since the positive D-D effect in " H present " is at least partly spurious, and in " H absent " the unbiased contrast is negative (though not significantly), the conclusion must be that the overall D-D effect, although shown as significantly positive, is in fact not significant and, so far as the evidence goes, possibly negative.

When the D-D effect is analysed in respect of the other two factors, *e.g.* F present and F absent, half of the bias passes to each component, exerting a positive effect on each. It is therefore a matter for no

TABLE III.

Analysis of Single Factors.

(Actual differences due to Factor (i) in presence and absence of Factor (ii).
Values in **bold** type are significant.)

Criterion	Factor (i)	H		S		D		F	
	Factor (ii)	+	—	+	—	+	—	+	—
Height of Haulms at 45 Days. (cm.)	H	—	—	**32**	—21	**92**	—21	**42**	**39**
	S	—5	**—58**	—	—	**49**	22	**25**	**56**
	D	25	**—88**	19	—8	—	—	**37**	**44**
	F	—30	**—33**	—10	21	**32**	**39**	—	—
	TOTAL	**—63**		11		**71**		**81**	
Weight of all Tubers. (gm.)	H	—	—	—55	78	**855**	—226	181	**674**
	S	**—613**	**—480**	—	—	**393**	236	**555**	300
	D	—6	**—1087**	90	—67	—	—	**388**	**467**
	F	**—793**	—300	139	—116	275	**354**	—	—
	TOTAL	**—1093**		23		**629**		**855**	

surprise that the component values with respect to S and F are all positive, some significantly and some not, under both criteria.

Fertilizer effect: F.

In the main fertilizer contrast, of the 4th numerical column of Table III, the four biasing treatments (containing both H and D) are distributed equally on the two sides of the contrast, as is the case also with the main sterilization effect. The contrast as a whole should, therefore, be unbiased.

The four biasing treatments appear, two each side similarly balanced, in " H present " and " D present," but not at all in " H absent " or " D absent," and one each side in both components: " S present " and " S absent." The four sub-contrasts which entirely exclude these anomalous treatments, namely " H absent " and " D absent " under the two criteria, are all significantly positive. This confirms the positive significance of the main effect.

Interactions.

The bias discussed above appears to have no effect on the interactions, except of course the HD interaction where, in the absence of any real interaction effect, the expression reduces to — 4(h). Since the effect of eelworm (H) is known to be negative, this bias will lead to a spurious positive tendency in the value for HD. The positive values in Table II under the Height criterion are, therefore, suspect, and should be disregarded, as in the case of the 1946 data.

The full analysis of Height is of doubtful significance, because the mean square for original treatments was not significantly greater than that for interaction between original treatments and fertilizer. The only significant interaction remaining to be considered is, therefore, HF under the weight criterion. Eelworm (H) is significantly negative and fertilizer (F) significantly positive; a significant negative interaction between them means that, where both factors are present, the yield is less than would be expected if the two factors acted independently.

The disturbing effect, if any, of the four anomalous treatments can be removed by measuring HF in the absence of D-D. In this case HF is still negative, but no longer significantly so. In the remainder (" D-D present ") the same is true, with the value of HF about 10% smaller than in " D-D absent ". The interaction effect is therefore probably real, but insufficient to show as significant when only half the pots are used.

The biological explanation of this significantly negative HF interaction probably lies in the fact that the fertilizer leads to an increased

root growth, and the roots support a larger population of eelworm cysts in the (hf) pots than in the (h) pots. Thus, although from the design of the experiment the (h) and (hf) pots are exposed on the average to an equal risk of eelworm infection, the (hf) pots can support a larger population of new cysts on the roots, and these are the cysts which are effective in reducing yield. The practical implication of this situation is that fertilizer dressings on badly infected soil will, to some extent, fail to be reflected in increased yields but instead will be used up in multiplying further the eelworm population.

This completes the discussion of the plant criteria, concerning which the following conclusions may be briefly stated. There is an entire reversal of the 1946 situation, in which eelworms had no effect, steam sterilization and D-D injection both had a positive effect and there was a negative interaction between them. In 1947 eelworm infection had a strong negative effect, neither steam sterilization nor (in spite of Table II) D-D injection showed any residual positive effect and their interaction also sank into insignificance. Apart from the expected positive effect of adding fertilizer, the only useful result got from this new factor was the revelation of a negative interaction between it and eelworm infection.

Eelworm Criteria.

In the preceding discussion of plant criteria, eelworm infection was one of the four factors whose effects on the plants were under examination. By using only the 40 pots containing eelworm, and making counts of eelworm cysts and larvae, it is possible to investigate the effects on the eelworm population of the three factors S, D, F, and their interactions.

Table IV gives the geometric mean counts of cysts and larvae for each of the eight treatments, based on soil samples taken at the conclusion of the 1947 experiment. Comparison with the corresponding Table IV of the previous paper (1946 results) shows that, while the cyst counts are roughly similar, the counts of larvae per cyst are greatly reduced ; those from pots treated with D-D vary from 1/5 to 1/3 of the previous counts, and those from the untreated pots are only about 1/10. There has plainly been a lethal factor affecting all the pots of soil, and this is thought to have been climatic. In the 1946/7 winter the pots were exposed to very low temperatures, sufficient to crack the walls of several. During the 1946 growing season very large pots had been used ; these had been waxed internally ; the season was normally wet and there was no difficulty in keeping the soil from drying out. During the 1947 season on the other hand, the pots were much smaller

and unwaxed, and the season was hot and dry. It seems likely that high temperature alone may have been responsible for the death of so many larvae.

TABLE IV.
Geometric Mean Counts of Eelworms.
(Based on 5 pots per treatment.)

Treatment	Cysts/gm. of soil (c)	Larvae/Cyst (l)	Larvae/gm. of soil (g)
(h)	2.62	7.57	20.0
(hs)	2.50	10.6	26.8
(hd)	1.44	0.333	0.483
(hsd)	1.73	0.561	0.972
(hf)	2.93	6.18	18.3
(hsf)	2.04	11.8	24.2
(hdf)	1.22	1.47	1.81
(hsdf)	1.44	0.668	0.968
Mean :	1.90	2.39	4.59
V :	2.83%	23.7%	51.4%

TABLE V.
Effect of Factors : Eelworm Ratios.
(Ratios of 20 pots with, to 20 without the factor.)
Values in **bold** type are significant.

Factor	(c)	(l)	(g)
S	0.983 (1.02)	1.20	1.18
D	**0.578** (**1.73**)	**0.0748** (**13.4**)	**0.0432** (**23.2**)
SD	**1.209**	0.731 (1.37)	0.884 (1.13)
F	**0.896** (**1.12**)	1.48	1.32
SF	0.919 (1.09)	0.778 (1.29)	0.715 (1.40)
DF	0.939 (1.07)	1.55	1.46
SDF	1.075	0.668 (1.50)	0.719 (1.39)

Whatever the cause, the 80 pots had been freely randomized for position and the effects of treatments might be expected still to appear, though somewhat masked by the lethal factor. Table V shows the effect of each of the seven factors, the differences (such as those of Table II) in the logarithmic totals becoming ratios when re-transformed. Ratios less than unity have been quoted also in reciprocal form (in parenthesis) for ease of comparison. It will be seen that the effects are broadly similar to those of 1946. There is one new feature, a significant positive SD interaction under the cyst-count criterion, and a just significant negative fertilizer effect, also under cyst count, but otherwise the only significant factor is the negative D-D effect.

TABLE VI.

Analysis of Single Factors. (Actual differences of logarithmic counts due to Factor (i) in presence and absence of Factor (ii). Values in **bold** type are significant.)

Criterion*	Factor (i)	S		D		F	
	Factor (ii)	+	—	+	—	+	—
Coded log. (Cysts/200 gm.)	S	—	—	**—156**	**—321**	**—84**	—11
	D	**75**	**—90**	—	—	**—75**	—20
	F	—44	29	**—266**	**—211**	—	—
	TOTAL		**—15**		**—477**		**—95**
log {(Larvae/8 cysts)+1}	S	—	—	**—12.6**	**—9.90**	0.60	**2.78**
	D	—0.58	2.14	—	—	**3.60**	—0.22
	F	—0.31	1.87	**—9.35**	**—13.2**	—	—
	TOTAL		1.56		**—22.5**		3.38

* For convenience, cyst counts were coded as: 100 (log x—2); and larval counts as: log (x+1) to avoid zeros.

This is found under all three criteria, as in 1946, but is less in magnitude under larval counts than in 1946, since the unknown lethal factor had a proportionately larger effect on larvae in pots not injected with D-D.

It is possible to analyse the effects of single factors under eelworm criteria, as was done in Table III for plant criteria. This, shown in Table VI, scarcely warrants detailed discussion since, apart from the indisputable D-D effect, which is significantly negative under all criteria both in the absence and presence of other factors, the only significant effect is the negative effect of fertilizer on cyst counts. It is interesting that this is significantly negative only in the presence of either S or D ; in their absence it is still negative but not significantly so. There would seem to be no simple explanation of this curious and unexpected result unless, being only just above the 5% probability level, it is, in fact, a spurious random effect. There is no sign of it in the larval counts, where there is a positive effect due to F which almost achieves significance.

The positive SD interaction, under cyst counts, is accompanied by the significantly negative D-D effect and a slight negative sterilization effect. In 1946 both these factors had a positive effect on the plant criteria, and a negative interaction indicated mutual interference when they were present together. The present positive interaction again points to mutual interference in their negative effects on cyst counts, and may be considered as a reflection of this previously established point.

Summary of Observations.

Taking into consideration the whole of this experiment, as reported in this and the two preceding papers, the following are the salient conclusions.

1. Of the various plant criteria tested, the height of plants at about the 45th day after planting, and the weight of all tubers produced, are the two most useful criteria. Eelworm criteria are conveniently analysed in logarithmic transformation.

2. In the first season the presence of numerous cysts of *Heterodera rostochiensis* had no detectable effects on the growth or yield of the potato plants. Plants grown in the same soil without further treatment in the second year both were shorter and produced a much smaller crop than the uninfected.

3. The steam-sterilization of soil had outstanding positive effects on height and weight in the first season, but there was no trace of this in the second season. There was slight but inconclusive evidence

that steam-sterilization led to an increase in cyst density in the first season and a decrease in the second.

4. Injection of D-D, at a rate roughly corresponding to 1,460 lb./acre, had no significant effect on the height of plants in the first season (except in the presence of eelworm, where the effect must be ascribed to its nematocidal action), but considerably increased the crop yield, even in the absence of eelworm. This, the soil-amendment effect, was about 28% of the corresponding sterilization effect.

In the second season there appeared to be a residual positive effect of D-D, both on height and on weight, but further analysis showed that this occurred only in the presence of eelworm (in its absence, the effect was negative under both criteria). This is interpreted as a mere after-effect of the original nematocidal action. The latter was very great in the eelworm criteria of the first season, and its effect was still very prominent in the second.

5. The addition of artificial fertilizers as a new factor in the second season had the expected positive effect on height and weight, and a just significant negative effect on cyst count.

6. In the first season the plant criteria showed a negative SD interaction, the positive effects of sterilization and D-D not being independently additive. In the second season this had disappeared from the plant criteria, but under cyst counts there was a positive SD interaction, the separate factors being here negative. In both cases the evidence points to mutual interference between these two factors.

7. In both seasons, a positive HD interaction under the height criteria is interpreted as an inverted form of a negative eelworm effect, produced owing to the very marked nematocidal action of D-D in the first season.

8. The only other interaction of interest was HF, significantly negative under yield in the second season. This is taken to mean that, in heavily infected soil, a part of the beneficial effects of using artificials is wasted : the enhanced root growth is to some extent utilized in producing more eelworm cysts rather than more tubers.

Discussion.

The failure of *H. rostochiensis* to exert any detectable influence on potato plants in pots, in the first season, was noted by Buckhurst and Fryer (1931) in the case of steam-sterilized soil, and was confirmed by Carroll (1933) in some of his series of trials. In both cases, the protective action of steam sterilization no longer obtained in the second season (Carroll and McMahon, 1935). Buckhurst and Fryer tentatively

postulated a soil factor combining with eelworm infestation to produce potato sickness, this factor being neutralized temporarily by partial sterilization and being concerned with the early nutrition of the plant. On the other hand, Carroll and McMahon (1935) showed that recent steam sterilization had a neutralizing effect on the potato-root diffusate which normally stimulates the hatching of eelworm larvae: cysts added to sterilized soil produced about the same number of larvae, but only after a delay of up to six weeks—the delay in hatching being inversely proportional to the time elapsing between sterilization and planting. After a lapse of six months there was little difference between sterilized and unsterilized soil. Correlating with this, Carroll and McMahon found that they could produce disease symptoms in pots if hatched larvae were watered in as soon as the potato sprouts appeared, but not if they were added only after the shoots were several inches high, though in both cases cysts were later abundant on the roots. Finally, cysts added to recently sterilized soil could be made to produce full disease symptoms if the potatoes were watered with root diffusate instead of plain water. (This was not confirmed by Carroll and McMahon, 1937.) The whole situation indicated that disease could be correlated with degree of infestation (up to an optimum of about 4 cysts per c.c. of soil), except where hatching was for any reason delayed; in this case the young plant got away and made a good root-system, and disease symptoms failed to appear even if the roots later became smothered in cysts.

The effect of steam sterilization in delaying hatching is not the whole story, since in the present experiment its beneficial effects on the height of haulms and weight of potatoes were only slightly less in the absence of eelworm than in its presence. It would thus seem that steam sterilization both delays the hatching of eelworms beyond the dangerous early period of potato growth, and at the same time stimulates the plant to make a good root system. This is borne out by the results obtained by Buckhurst and Fryer and by Carroll in steam-sterilized soil to which no eelworms had been added, and is in agreement with the generally acknowledged results of partial sterilization. The combination of these two effects, however, is likely to mean that, although the potato plants grown in recently sterilized and re-infected soil will escape eelworm disease, they will lead to an increase in the eelworm population of the soil (unless this has already reached its optimum value). Thus, in the present experiment, there is evidence of a slight increase in the cyst and larval populations in steam-sterilized soil, at the end of the first season.

In the commercial sphere, potato fields are neither steam-sterilized nor massively re-infected with cysts, and the above argument would seem to have no practical application, experimentation apart. It is claimed, however, that the injection of D-D has a soil amendment effect somewhat similar to that of steam sterilization, in addition to its nematocidal action. Carter (1943, 1945) working mainly with pineapples in Hawaii, claimed a soil amendment effect from D-D in addition to its nematocidal action, and Tam (1945) has explained the former in terms of a destruction of nitrifying bacteria, the pineapple assimilating ammonia-nitrogen (from ammonium sulphate dressings) in place of nitrate-nitrogen, and making better growth with this type of metabolism. The direct assimilation of ammonium salts by plants was discussed and experimentally verified by Hutchinson and Miller (1909, 1911) of Rothamsted, and concurrently the partial-sterilization effects from steaming and from various volatile substances were investigated at Rothamsted by E. J. Russell and co-workers (a useful short list of references is in Russell and Buddin, 1914). The present experiment has demonstrated a soil-amendment effect from D-D, even in the absence of eelworm, as well as a strong nematocidal effect, after using high rates of dosage. In the absence of eelworm the soil-amendment effect was found in yield of tubers but not in height of haulms; this difference may well be associated with the known phytocidal action of D-D for some time after injection. Thus, the effects of D-D are complex and difficult to disentangle. In its effects on plants, there is an early phytocidal action which, when it does not kill, retards growth; this is later counterbalanced by the soil-amendment effect which stimulates growth. In its effects on eelworm there is certainly a marked nematocidal action (if high enough dosages are used), but it is not yet clear what happens to those eelworms which survive. Their normal physiology may well be disturbed, for a time at least. In addition, the delayed hatching noted by Carroll for steam-sterilization (through some interference in the root-diffusate stimulus) may also apply for partial sterilization by D-D. These are matters for further investigation.

The present experiment has been largely exploratory. It has established useful criteria and methods, and has revealed some interesting effects from the main factors and their interactions. The attempt to interpret the results has at least shown the great complexity of the whole situation when a substance like D-D is used with the object of killing eelworms. Some eelworms are killed and potato plants make improved growth, partly through the killing of eelworms and partly

through the soil-amendment effect. The improved growth includes a better root system which in turn is able to support a larger population of eelworms, drawn from the unkilled survivors. The final situation will be a resultant of many factors, chief among which will be the dosage of D-D used. In the present experiment, where a heavy dosage was used, the eelworm population was greatly reduced by the end of the first season. In other experiments, to be published elsewhere, a lower dosage led to a slight kill followed by a larger increase so that the final situation was a larger population of eelworm than in untreated controls. In such a case D-D may ward off eelworm disease in the ensuing potato crop, and lead to increased yields, but only at the cost of a build-up in the eelworm population. From this point of view, in relation to rotations of non-susceptible crops, it may be better to inject D-D immediately *after* the potato crop, and sacrifice the soil-amendment effect.

Biologically, it is the usual situation of a dynamic balance of factors. The establishing of an equilibrium, most favourable to the potato in the long run, is in its nature a long-term problem, for the solution of which many data are still lacking.

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Tuber-rot Eelworm of Potato and its Weed Hosts.

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In the British Isles potatoes are subject to many pests and diseases of animal, fungal and virus origin. In the first class there are two different kinds of eelworms or nematodes which attack the plant, one affecting the roots and the other the tubers. The former is the cyst-forming species *Heterodera rostochiensis* and the latter was for many years considered to be a specialized race of the stem eelworm, *Anguillulina dipsaci*. As a result, however, of work carried out by Thorne (1945) in the U.S.A., it is now known to be a distinct species, *Ditylenchus destructor*. Though resembling *A. dipsaci* closely in size, general shape and structure, *D. destructor* differs from it in one or two

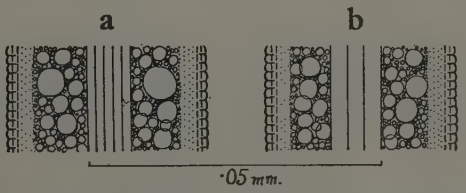

Fig. 1.—Surface view of a small portion of the body of eelworms under high magnification. The lateral field is in the middle of each with the longitudinal incisures. (a) *Ditylenchus destructor*, (b) *Anguillulina dipsaci*.

points of microscopic detail which are constant and can be seen in suitably mounted specimens when examined under high magnification. The most important of these is the presence of 6 fine longitudinal lines or incisures on each side of the body in the area known as the lateral field as compared with 4 such lines in the case of *A. dipsaci* (fig. 1).

The diseased tubers (Plate I) look rather like those attacked by "blight" (*Phytophthora infestans*), but whereas these are firm to the touch those infected with *D. destructor* have a soft feel and a slightly sunken cracked surface. The diseased tissue is mealy and is usually confined to the outer parts of the tuber.

Plot experiments have, in the past, failed to reveal any other crop plant of economic importance as a host of the tuber-rot eelworm; see Goodey (1935). In that paper also results were presented which indicated that even when plots were cropped year after year with potatoes the degree of eelworm infestation in the tubers lifted decreased

rather than increased. As a consequence it has always been difficult to account for the persistence of the parasite in fields unless there were some other host plant, such as a perennial weed, on which it could live in the absence of the potato. Such a host plant has now been found. In Prince Edward Island, where the parasite was found a few years ago infesting potato tubers, Hurst (1948) has reported that Corn Mint, *Mentha arvensis* L., can serve as a weed host.

We have carried out a pot experiment in which we planted clean runners of Corn Mint, *M. arvensis*, in sterilized soil mixed with parings of potato tubers affected with *D. destructor*. By this means we were able to confirm the transference of the parasite from potato to the mint rhizomes in which brown lesions were produced (Plate I). In the discoloured tissues just below the surface of such lesions we found adults and all developmental stages of *D. destructor* ; the adults showing the characteristic 6 incisures on the lateral fields. We have also made observations in fields in the Fens where in 1947 potatoes were affected with tuber-rot eelworm and where, this year, 1948, wheat was grown. Through the good offices of Mr. B. A. Cooper, Advisory Entomologist, Agricultural Institute, Kirton, Boston, Lincs., two such fields, some miles apart, were visited in late August after the wheat had been cut and carried. In both fields Corn Mint, *M. arvensis*, was found to be a very abundant weed. Another common weed was Corn Sowthistle, *Sonchus arvensis* L. Many specimens of both weeds, with their creeping underground stems, were dug up and brought to the laboratory for detailed examination. Brownish lesions were present on the rhizomes of both but were more plentiful on those of the former and within these lesions *D. destructor* was found. The presence of the 6 incisures on the lateral fields of males and females was confirmed. The occurrence of the living parasite in the rhizomes of these two weeds almost twelve months after the lifting of the previous year's potato crop, proves that they can serve as alternative reservoir hosts of the parasite. *Sonchus arvensis* L. is a hitherto unrecorded host of *D. destructor*. Further investigations on the host relationships of the parasite are in progress in this department.

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Plate I.—Above : Potato tuber attacked by *Ditylenchus destructor*. Left : Surface view showing typical advanced symptoms. Right : Longitudinal section showing rot round periphery. Nat. Size. Below. Left : Rhizome of Corn Mint showing lesion extending between nodes. Right : Longitudinal section of another rhizome with rot extending into tissues of internode. x 4.

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A Quick Method of Demonstrating Nematodes of the Genus *Aphelenchoides* in Leaves.

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When the presence of nematodes in plant tissues is known or suspected it is often useful to be able to show the exact situation of the parasites and their biological condition, *i.e.* whether they are quiescent or active. If plant tissues are teased apart in water active nematodes will be freed and will soon be seen swimming in the water, or, if they are in a state of quiescence, and yet viable, they will revive in an hour or more and become active in the water. Whichever is the case, there may occasionally be some doubt as to whether the nematodes were really in the tissues of the plant or were carried on the outside. The larvae and eggs of most parasitic eelworms are so small that they may easily be overlooked amongst the teased plant tissue, with which there may also be numbers of detached plant hairs closely resembling nematodes in their shape, size and transparency. If the parts of the plants likely to contain eelworms can be stained so that the worms take up the stain in contrast to the plant tissue, and can be seen in their natural position, a much more accurate picture of the situation may often be obtained than that derived from teasing the tissues in water.

In 1937 Goodey described how cotton blue or acid fuchsin may be used in lactophenol for staining nematodes in plant roots. He found this method unsatisfactory for use on shoot structures and he recommended scarlet R in 70% alcohol, finding it particularly effective in demonstrating *Anguillulina dipsaci* in clover and oat seedlings. When he tried the method on a narcissus leaf the worms were satisfactorily stained, but the leaf was rather too thick for them to be clearly seen. Scarlet R was also successfully used for staining *Aphelenchoides ritzema-bosi* in a chrysanthemum flower-head, but better results were obtained with strong Flemming's solution used according to the method given by Godfrey (1935).

In an attempt to find out something of the biology of *Aphelenchoides ribes* in the blackcurrant the present writer wanted to stain the nematodes *in situ* in the currant buds and tried both the scarlet R and Flemming's solution methods. Neither proved to be satisfactory, the worms not being stained sufficiently to be seen easily amongst the plant tissues. With Flemming's solution many of the worms were

merely stained a pale grey instead of the dense black which was expected. Attempts to stain *A. ritzema-bosi* in chrysanthemum leaves were also unsatisfactory.

Of various other stains tested only one seemed satisfactorily to permeate the plant tissues and stain the nematodes in a fairly short time, and this was a solution of acid fuchsin in lactophenol, used boiling.

The leaves to be stained may be either fresh or dried. If they have been dry for some time it is advisable to soak them in water for an hour or two to allow the worms, which may be rather shrunken, to swell to their full extent. They will, nevertheless, stain quite well in the dry state. No advantage was observed in first killing fresh leaves by plunging them into boiling water. Large chrysanthemum leaves may be treated whole or cut into smaller pieces.

The selected plant material is plunged into boiling lactophenol in which is dissolved 0.1% to 0.5% of acid fuchsin, and the solution kept gently boiling for about five minutes, thicker leaves requiring longer than thinner ones. The leaves are left in the solution while it cools for half to one hour according to how rapidly they take up the stain. To determine whether the leaves are sufficiently stained they may be removed from the lactophenol solution and the surplus stain washed off in running water. If they are then held up in a good light the progress of the staining can be seen. If they show unstained patches they can be replaced in the stain for a further period until it has permeated the whole leaf. They are then taken out, washed in running water for a minute and placed in 50% alcohol, which slowly removes the stain. The leaves should be left in it until nearly all the red colour has come out, but if they are left in it too long, *i.e.* for several days, the colour will be removed from the nematodes as well as from the plant tissue. If the leaves are very darkly stained boiling 50% alcohol will be found to remove the colour more rapidly than cold.

From 50% alcohol the leaves are transferred to liquid phenol. This was found to be a very effective clearing agent as, not only does it clear even thick chrysanthemum leaves within an hour, but it also removes a great deal of the green colour of the leaf which is still present at this stage. After half to one hour in phenol the leaves may be examined under a binocular microscope and any nematodes present should be clearly visible, stained red in the but lightly stained plant tissue. The leaf veins often retain more colour than the inter-veinal tissues, but they can easily be distinguished from the eelworms.

As phenol becomes somewhat brown on exposure to light it is

better to transfer the leaves to clear lactophenol if it is desired to keep them for more than a day. For permanent mounts suitable pieces of tissue containing nematodes may be cut out from the leaves, transferred through 50% and 70% alcohols to iso-butyl alcohol, and from there mounted on slides in euparal. The lactophenol gum mounting medium described by W. H. Davis (1924) has been tried, as its use would avoid the necessity of taking the material through the alcohols, but the stain was found to come out of the material into the medium in the course of a week or two.

The foregoing method, modified according to the thickness of the plant material being processed, has satisfactorily shown up *Aphelenchoides ritzema-bosi* in both fresh, green and dried, brown chrysanthemum leaves. This parasite, in various stages, including eggs, was also clearly demonstrated in dahlia leaves taken straight from the plant in October. In this case numbers of worms were seen in parts of the leaf which had appeared fairly healthy, and the parasite was evidently actively feeding and reproducing. In dry, brown chrysanthemum leaves the worms were tightly coiled, and had mostly taken up a position close beside the main veins. Dried leaves of *Lilium Henryi* and *L. regale* were stained and shown to contain enormous numbers of nematodes in all stages of development. On examining unstained specimens these were found to be *A. olesistus*. Though many of the worms in the leaves were dead they took up the stain as well as the living ones. Strawberry leaves, blackcurrant leaves and fern (*Pteris* sp.) leaves were stained in the manner described and, although they contained no nematodes, they appeared to be penetrated by the stain, and were cleared quite satisfactorily. There seems to be no reason why the method should not be used successfully on most plant tissues.

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*Notes on some Avian Species of *Ascaridia*.

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The present study is based on a large collection of bird ascarids which was placed at the disposal of the writer for examination. Most of the material came from birds which died at the London Zoological Gardens during the years 1925 to 1939 and in 1946, while the remainder consisted of specimens sent to the Department of Parasitology, London School of Hygiene and Tropical Medicine from various parts of the world. Though it is not possible to ascertain whether infection of the hosts with the parasites took place before or after their arrival at the Gardens, the original host-localities are indicated in each instance. The collection was found to comprise six species and a new variety, namely : *Ascaridia galli* (Schrank, 1788) Freeborn, 1923 ; *A. columbae* (Gmelin, 1790) Travassos, 1913 ; *A. columbae* var. *vinagori* n.var. ; *A. cristata* (Linstow, 1901) Railliet and Henry, 1914; *A. compar* (Schrank, 1790) Travassos, 1913 ; *A. hermaphrodita* (Froelich, 1789) Railliet and Henry, 1914, and *A. numidae* (Leiper, 1908) Travassos, 1913. Though no new species were found, some morphological characters were observed which are of considerable interest and may throw some light on the systematic relationship of the species comprising this genus. New morphological characters were also found which either disagree with or supplement previous descriptions of certain species.

A. galli (Schrank, 1788) Freeborn, 1923,
and *A. styphlocerca* (Stossich, 1904) Railliet and Henry, 1914.

Hosts : Peachieta rehore, Spilopelia suratensis, fowls, and guinea fowls.

Habitat : Intestine.

Localities : India, Malaya, Java, Palestine, Egypt, E. Africa, S. Africa, West Indies and England.

There were twenty-four collections comprising 536 specimens of which 132 (91 males and 41 females) were examined. The majority

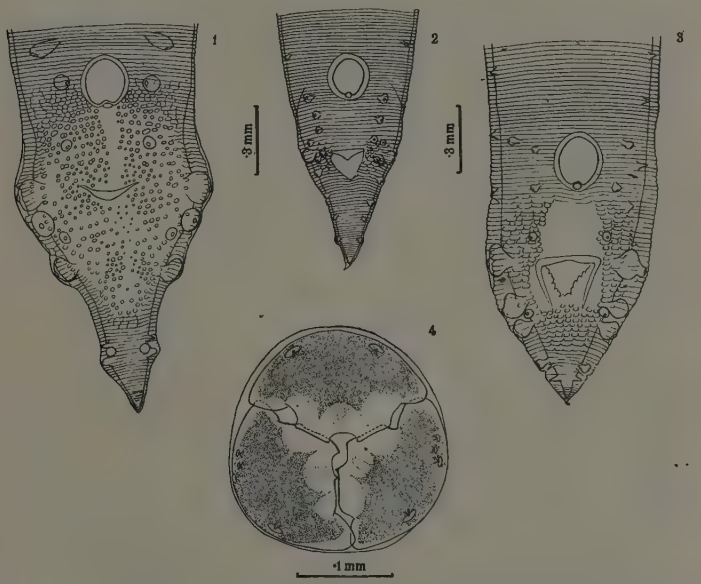
* Part of a thesis approved by the University of London for the award of the Ph.D. Degree.

came from fowls in different parts of the world. All the specimens exhibited cuticular protuberances or tubercle-like bodies on the ventral surface of the male tail. The appearance and distribution of these bodies suggest that they are derived as a result of the interruption of the cuticle between the transverse striations of the body. As shown in Figure 1, this interruption begins in a moderate degree at the region of the preanal sucker; it is more marked posteriorly where there is transformation into numerous isolated, tubercle-like bodies, ovoid or round in shape, varying in size, and apparently irregularly distributed over the ventral surface of the caudal region around the anus. More posteriorly the normal striations are resumed in the subterminal region of the tail. These protuberances are seen not only in the adult male but also in immature specimens, thus suggesting that it is a specific character of *A. galli*. In this connection it should be noted that there is a similar interruption of the transverse striations in the caudal region of the male of *A. cristata*. In this species (Fig. 3), however, the transformation is less well developed and exhibits a smaller number of rhomboidal or triangular bodies which still retain the outline of the transverse striations. They are regularly and evenly distributed, like the tiles on a roof, over the ventral surface of the tail, thus presenting an appearance quite distinctive from those in *A. galli*. In the male of *A. columbae*, *A. compar*, *A. hermaphrodita*, and *A. numidae*, the cuticle in the caudal region shows uninterrupted transverse striations with the exception of the adanal area where there is some evidence of the slight cuticular interruption as is seen in Fig. 2. So far as the present material is concerned, it can be concluded that the genus *Ascaridia* exhibits three types of tail ornamentation. In one the tail is covered with the cuticular tubercle-like bodies, only seen in *A. galli* (Fig. 1). In another the cuticular tubercles are either absent or very faintly developed, but only in the adanal region, as in *A. columbae* (Fig. 2). Between these two extremes there is an intermediate type as seen in *A. cristata* (Fig. 3).

Chiefly on the presence of this character (tubercle-like bodies) in the male caudal region Wu and Kung (1944) created a new species, *A. sinensis*, for an ascarid collected from the chicken at Chungking, China. It is now evident that this species is a synonym of *A. galli*, not only because this character is common to both, but also on account of their similarity in other essential features.

As regards the synonymy of *A. galli*, the present writer agrees with Baylis's contention that all the species of *Ascaridia* mentioned in his paper are synonymous with *A. galli*. Nevertheless, there are still some

divergent opinions (Neveu-Lemaire, 1936 and Sprehn, 1932) on this point. *A. lineata* is still regarded by some authors, e.g. Cram (1927) and Neveu-Lemaire, as a distinct species on the grounds that *A. galli* possesses longer spicules (over 1.9 mm.), a larger preanal sucker (over 0.217 mm.), the presence of slender lateral body alae and only two pairs of subterminal caudal papillae instead of three in the male tail.



1. *Ascaridia galli*, ventral view of male tail. 2. *A. columbae*, ventral view of male tail. 3. *A. cristata*, ventral view of male tail. 4. *A. columbae*, end-on-view of anterior end.

The writer's examination of the material from China, India, Palestine, Egypt, E. Africa, S. Africa, West Indies and England, shows that the spicules vary in length from 1.33 to 2.424 mm. and the sucker (outer rim) measures 0.152 to 0.248 mm. in longitudinal diameter. Moreover, no lateral alae were seen. All these findings confirm those of Ackert (1931). The subterminal caudal papillae in the male consist

of three pairs instead of two though they are very often subject to variation in size and position. The ventral subterminals, in particular, are sometimes so small or so close to their neighbours in some individuals that they may easily escape notice, and the first subterminal pair are sometimes so slender that they are easily mistaken for cuticula ridges. The writer is therefore inclined to believe that the lack of a pair of papillae in the subterminal group as illustrated by some previous authors, is probably due to an oversight. The tubercle-like bodies present on the ventral surface of the male tail mentioned earlier in this paper as a specific character, are present in all the specimens in question, irrespective of host differences and country of origin, and are therefore of specific value in the present instance. From the evidence presented here it is concluded that the treatment of *A. lineata* and *A. galli* as distinct species, cannot be justified.

A. perspicillum (Rudolphi, 1803), is still regarded by Sprehn as different from *A. lineata* in that it possesses only two pairs of subterminal papillae in the male tail and the dorsal oral lip is larger than either of the submedians. The first point has just been discussed. As to the second point, the dorsal lip is usually a little larger than the submedians in the present material and so the difference here is merely one of degree.

As regards *A. granulosum* (Linstow, 1906), nothing further can be added to Baylis's observation and conclusion that this species is also a synonym of *A. galli*.

The present collection included ten ascarids from the fowl in Uganda. These had been identified previously as *A. styphlocerca* (Stossich, 1904). The examination of these, however, shows no significant departure from the characters of *A. galli*, but on account of the exaggerated dorsal curvature of the male tail, it could not be ascertained whether the first pair of subterminal caudal papillae is present or not. Unfortunately the writer has not had access to the original specimens of Stossich, but the figure of the caudal extremity of the male as shown by Stossich is like *A. galli* in essential features. According to this there are three pairs of preanal papillae, two on either side of the sucker region and one about midway between the sucker and cloacal opening. There are five pairs of postanals of which one unpaired papilla is situated between the last two pairs on the right side, and a medium one between the members of the last postanal pair. Of the first three postanal pairs the third is rather ventral in position. According to Cram (1927) a single male specimen from S. Africa was very much like that figured by Stossich except that the median papilla

figured between the last postanal pairs was absent and the second from the posterior end figured as an asymmetrical papilla, was a symmetrical pair, making up six pairs of postanals, five of which were lateral. As a result of examination of the specimens labelled "*A. styphlocerca*" in the present collection the writer agrees with Cram's findings. In some specimens of *A. galli* there is a round vacuole-like body situated medially between the most posterior papillae. It is probable that this body is the same as that shown in Stossich's figure of *A. styphlocerca*. The refractive granulations over the ventral surface of the male tail figured by Stossich and described by Cram are believed to be the same as the cuticular tubercle-like bodies observed by the writer in *A. galli*, which have hitherto remained unmentioned. It seems probable that *A. styphlocerca* is synonymous with *A. galli*.

A. columbae (Gmelin, 1790) Travassos, 1918.

Hosts: *Columba guinea*, *C. leuconata*, *Leucosarcia melanoleuca*, *Phaps chalcoptera*, rock pigeons and doves.

Habitats: Intestine and body cavity.

Localities: Australia, India, Himalayas, W. Africa, E. Africa, England and North America.

There were sixteen collections comprising 219 specimens of which 67 (49 males and 18 females) were examined. The study of specimens of *A. columbae* from both pigeons and doves of different species and even different genera shows some new features which do not conform with the descriptions of previous authors. Firstly, as shown in Fig. 4, the subventral lips of the mouth are each provided with four papillae, one being larger and slightly submedian in position, the other three rather small and grouped in the subdorsal position. Secondly, the anterior extremity of the worm is provided with alae which extend backwards on the lateral lines for a distance of 2.26–2.85 mm. from the anterior end, with a maximum width of 0.148–0.22 mm., but about eight pairs of papillae are situated within them instead of two or three as noted by Baylis and Daubney (1922). Posteriorly to them, on the lateral line of the body there is a series of paired papillae of which the exact number could not be counted owing to extreme curvature of the body. Thirdly, the spicules are alate averaging 2 mm. long by 0.082 mm. wide as shown in Fig. 6. Lastly, as noted by Baylis and Daubney (1922) the caudal papillae are subject to variation in position and number. Unlike their specimens, however, the typical

number of papillae consists of twelve pairs in the present material, namely, four pairs of preanals, adanals and postanals respectively, as shown in Fig. 2, as against the fourteen pairs mentioned by them. In a good many individuals there is an additional pair of slender papillae between the last two postanals (subterminal group) making five postanal pairs and thus totalling thirteen pairs. The preanals are the most variable in number and position. Counting from the anterior, the first pair may be either immediately in front of, or at the level of, or even behind, the anterior rim of the preanal sucker, and occasionally one member of this pair is missing. The second pair is usually at the level of the middle of the sucker or sometimes at the level of its posterior end. In the specimens from *Phaps chalcoptera* the fourth pair, just in front of the cloacal opening is sometimes duplicated, making five preanal pairs. Of the four adanals one pair is especially large and situated laterally while the other three are smaller and variable in position.

A. columbae var. *vinagori* n. var.

Host: *Vinago delalandii*.
Habitat: Intestine.
Locality: Gold Coast.

	Female.	*Male.*
Body dimensions	43.54–43.81 mm. × 1.23–1.32 mm.	36.50–38.45 mm. × 1.06–1.16 mm.
Oesophagus dimensions ...	2.3 × 0.45 mm.	2.36–2.49 mm. × 0.366–0.411 mm.
Nerve ring from anterior end	0.64 mm.	0.657–0.73 mm.
Preanal sucker (outer rim)	—	0.229–0.251 mm.
Spicules	—	1.714–2.06 mm.
Vulva from anterior end...	20.77–21.1 mm.	—
Tail	1.436–1.715 mm.	0.549–0.571 mm.

There were two collections comprising 130 specimens of which twenty (15 males and 5 females) were examined. The specimens in question are recorded for the first time from *Vinago delalandii*, which is also the host of *A. fasciata* Baylis. They resemble *A. columbae* very much in essential features, especially the circumoral lips, the spicules, and the caudal papillae of the male, but they differ from it in some important points. Firstly, the cervical alae are 4.76–5.591 mm. long by 0.33–0.38 mm. wide, twice as long and wide as those of *A. columbae*. Secondly, the vulva is constantly a little posterior to the middle point

of the body, whereas it is usually in the middle of the body of *A. columbae*. Thirdly, the caudal papillae of the male are more variable in number and position, even in the specimens from the same individual host. These characters, although insufficient for the creation of a new species, are considered adequate for regarding the specimens as a new variety of *A. columbae*.

A. cristata (Linstow, 1901) Railliet and Henry, 1914.

Host: *Balearica pavonina*.
Habitat: Small intestine.
Locality: Sudan.

This material consists of a single collection of eleven specimens. They agree in essential features with previous descriptions of *A. cristata*, but there are some minor points of difference. Compared with the material described by Baylis and Daubney (1922), the present specimens are much longer, the female being 63–96 mm. long by 1.35–1.56 mm. wide, and the male 52–56 mm. long by 1.09–1.19 mm. wide (female and male being 38–40 mm. and 35–38 mm. long respectively according to Baylis and Daubney). In consequence, the well-developed cervical alae are much longer, extending backwards for a distance of 2.24 and 2.00 mm. from the anterior end in the female and male respectively (1.22 mm. long from the anterior end according to these authors), and usually terminate abruptly in front of the posterior end of the oesophagus. There is a series of paired papillae running throughout the whole length of the body on the subdorsal line, the first pair being situated at a distance of about 0.95 mm. from the anterior end. The distance between each pair of papillae in the cervical region is about 0.22 mm. but increases to about 0.72 mm. behind this region and persists until the caudal region is reached, where the distance between them decreases again. The caudal papillae of the male are twelve pairs in number as shown in Fig. 3. It is noticed that in addition to the caudal papillae there are on its lateral sides slender papillae which are a continuation of the body papillae series. It has already been mentioned in the discussion on *A. galli* that the ventral surface of the male tail of *A. cristata* is covered with rhomboidal cuticular protuberances which, however, are different from these found in *A. galli*. The spicules are alate, 1.05–1.08 mm. long by 0.013 mm. wide.

A. compar (Schrank, 1790) Travassos, 1913.

Hosts: *Alectoris graeca* and *A. graeca chukar.*
Habitat: Intestine.
Locality: India.

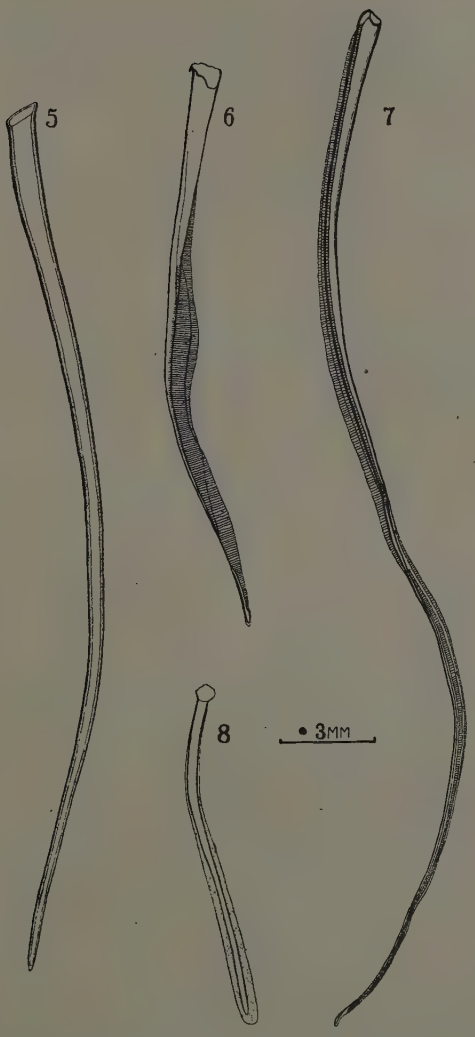
There were five collections comprising 225 specimens of which 44 (35 males and 9 females) were examined. The specimens examined were found to agree in essential features with *A. compar*, but they differ from it in some details. The specimens came from the same country (India) as those of Baylis and Daubney (1922), but they are much smaller, the adult females and males being 32.59–52.00 mm. and 20.15–34.50 mm. long respectively, whereas the female and male are 84–96 mm. and 36–48 mm. long respectively in Baylis and Daubney's material. On the dorsal lip of the mouth there are two small submedian papillae instead of a single central one. The spicules are 2.19–2.57 mm. long. As described by Baylis and Daubney there are ten pairs of caudal papillae in the male, but the ventral pair between the last two subterminal ones is not a constant feature of the present specimens.

A. hermaphrodita (Froelich, 1789) Railliet and Henry, 1914.

Host: Parakeet.
Habitat: Intestine.
Locality: British Guiana.

Two collections were available for examination. In general the material agrees with descriptions of *A. hermaphrodita*, particularly that of Travassos (1930). The mouth is provided with three strongly developed lips of which the dorsal lip is characteristically provided with two club-shaped lappets situated anteriorly and medially. No distinct lateral body membranes were seen though Froelich and Schneider (see in Cram, 1927) have described them in their specimens.

In the male the caudal papillae vary in arrangement and number. Generally speaking, thirteen pairs is the typical number. There are seven preanal pairs, the anterior two of which are near the sucker, and of these the second pair is very small. The other five are situated between the sucker and the cloacal opening and arranged in two rows on each side, one being ventral and the other lateral in position. There are six postanal pairs, of which two are in the subterminal region. Sometimes the first postanal pair, which is very close to the second large pair, is duplicated on the right side. There are no papillae



5. *Ascaridia compar*, spicule. 6. *A. columbae*, spicule. 7. *A.numidae* spicule. 8. *A. cristata*, spicule.

immediately behind the cloacal opening as shown in Travassos's figure. The spicules are about equal in size, 1.143–1.235 mm. long, but much shorter than those described by previous authors (2.30–2.93 mm. according to Travassos). The structure of the spicules is described elsewhere in this paper.

A. numidae (Leiper, 1908) Travassos, 1918.

Hosts: *Numida mitrata, N. calcarata* and *Guttera pucherani.*

Habitat: Intestine.

Locality: Africa.

There were six collections comprising 312 specimens of which 46 males and 10 females were examined. All the specimens examined are in agreement with the original descriptions of *A. numidae* except for the following points. Firstly, unlike Leiper's specimens the number and position of the papillae in the female tail are subject to variation. In addition to a pair at about two-thirds of the distance from the anus to the tip of the tail there are either one or two pairs more in front of them. Secondly, each of the subventral lips of the mouth is sometimes provided with an additional small papilla situated rather subdorsally.

Morphology of Spicules.

In the present study the examination of the spicules shows that in each species they have features of specific value. Moreover, they appear to be divisible into three types: (1) spicules without membranes, (2) spicules with wide membranes, and (3) an intermediate type having slender membranes. The first type is seen in *A. compar* as shown in Fig. 5. The second type is seen in *A. columbae* as well as its variety, in *A. hermaphrodita* and in *A. cristata.* In *A. columbae,* as shown in Fig. 6, two membranes, one apparently superimposed upon the other, run nearly throughout the whole length of the spicule. In fact, each runs along the edge of the spicule-body itself, one arising as a slender membrane a little before the other from its proximal end. As they extend backwards they become gradually widened to 0.082 mm. at the middle of the spicule and to 0.065 mm. at its distal end. In *A. hermaphrodita* the spicule is very similar to that of *A. columbae.* Contrary to the previous descriptions that the middle part of the spicule is enlarged to form an unilateral wing and its distal part is slender, two membranes, as in the spicules of *A. columbae,* arise from

the proximal end of the spicule and run along the edges throughout its whole length with a maximum width of 0.043 mm. at its middle point, so that the distal part of the spicule appears rather wide instead of being slender. No real projecting teeth were seen as shown in Schneider's figure. As regards the intermediate type it is conceivable that the membranes of the spicule vary in width with different species. In *A. numidae* as shown in Fig. 8, they are very slender, particularly at the distal third, and run throughout the whole length of the spicule. *A. galli* has spicules which are similar to those of *A. numidae*.

Summary.

1. A large collection of avian *Ascaridia* from a wide diversity of hosts and localities is reported upon. It comprised six species, *A. galli*, *A. columbae*, *A. compar*, *A. cristata*, *A. hermaphrodita* and *A. numidae*. Their morphology is examined critically with reference to previous descriptions, especially in the case of *A. columbae*, *A. cristata*, *A. hermaphrodita* and *A. compar*, and a new variety of *A. columbae* is described.

2. The taxonomic significance of spicule morphology and the tubercles on the ventral surface of the male tail in *Ascaridia* is discussed.

3. *Ascaridia sinensis*, *A. lineata*, *A. perspicillum*, *A. granulosum* and probably also, *A. styphlocerca*, are considered to be synonyms of *A. galli*.

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On the Species of *Diphyllobothrium* occurring in Birds, and their Relation to Man and other Hosts.

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In this paper it is intended to review the species of *Diphyllobothrium* occurring in birds and their relation to other hosts, and to attempt to determine the number of valid species. Recent experimental work has demonstrated that the adult stages of these parasites may occur not only in birds, but also in cat, dog and rat. Moreover, it appears from an examination of the morphology of new material from birds that man has been recorded as a host.

Baylis (1945) suggested that the number of species occurring in birds might possibly be reduced to two, namely: *Diphyllobothrium dendriticum* (Nitzsch, 1824) and *D. ditremum* (Creplin, 1825). The present work, which has been based on the study of a considerable amount of new material and of data obtained from the literature, tends to support this view. Further, it is apparent that two other species, one recorded as a natural parasite of man and the second obtained experimentally from a dog, are identical with *D. dendriticum*.

The writer wishes to express his sincere thanks to Dr. H. A. Baylis, who kindly suggested this investigation and provided the necessary material, and to Mr. S. Prudhoe for his valuable assistance. The writer's thanks are due also to Mr. H. W. Parker, the Keeper of the Department of Zoology, British Museum (Natural History), for kindly permitting this investigation to be carried out in his Department.

Material and Methods.

The material from naturally-infested hosts examined in the course of this work was collected by Dr. M. D. Hickey from aquatic birds, namely, *Larus marinus*, *L. argentatus*, *Phalacrocorax carbo*, *P. aristotelis*, and *Ardea cinerea*. These birds were obtained at the Poulaphouca Reservoir, Co. Wicklow, Eire, in 1944. Further material examined was obtained experimentally, and has already been reported upon, by Professor J. B. Duguid (1944) from dog and rat, by Dr. M. D. Hickey

and Mr. J. R. Harris (1947) from kitten, gulls and shag, and by Mr. K. Unsworth (1944) from puppies. All of this material appears to have been fixed in 5% formalin and most of the specimens are preserved in an extended condition, very few of them being contracted.

In addition to the above, the writer has, through the kindness of Dr. E. W. Price, of the United States Department of Agriculture, been able to examine American material from *Pelecanus erythrorhynchus*, *Mergus americanus* and a " gull " (all from Yellowstone Park Lake, Wyoming), *Larus californiensis* and a " gull " (Elk Lake, Wyoming) and *Larus delawarensis* (Washington, D.C.). Finally, through the kindness of Professor L. J. Thomas of the University of Illinois, a specimen of *Diphyllobothrium oblongatum* Thomas from *Larus argentatus* at Hat Island, Lake Michigan, has also been examined.

The worms show every degree of sexual development. Some have reached only the very early, almost larval, stages, while others possess fully-developed gravid segments. Measurements have been taken either from specimens stained with Mayer's paracarmine, or alum carmine, and mounted in Canada balsam, or from unstained specimens mounted in formalin. Serial sections were cut at thicknesses of between 16 μ and 20 μ, and double-stained with Ehrlich's haematoxylin and erythrosin.

Counts of the testes have been obtained from whole preparations, because in a few of the early mature segments, chiefly those in which the vitelline glands are not fully developed, the testes are plainly visible. However, to furnish a clearer picture of the arrangement of the testes, and to be more certain of their number, the cortical layer of the segments has been removed. This operation was performed on portions of strobila embedded in paraffin wax, and, under a binocular microscope, the cortical layer was gently scraped away with a scalpel or a microtome knife. After the wax had been dissolved with xylol, the objects were transferred to absolute alcohol, then to 80% alcohol and finally to 70% alcohol, after which they were stained with paracarmine.

Historical Remarks.

The publications on the species of *Diphyllobothrium* recorded from birds may be divided into two groups : those containing purely descriptive matter ; and those dealing with results of experimental work. In the first instance, several species have been described from naturally-infested birds ; in the second, authors have obtained mature worms by feeding plerocercoids to different final hosts (*e.g.* birds, dogs, cats and

rats) used for experimental purposes. As the result of the latter investigations there are to be found in the literature various descriptions of known species, or of species which are regarded as new. Apparently, all the plerocercoid larvae used in the experimental work were harboured by Salmonoid fishes (*Salmonidae* and *Coregonidae*).

The following eight species have been recorded from birds:

1. *Diphyllobothrium dendriticum* (Nitzsch, 1824). This species has been redescribed by Matz (1891) from a natural infestation, and has been obtained experimentally from a rat by Duguid and Sheppard (1944) and from dog, cat, *Larus marinus* and *L. argentatus* by Hickey and Harris (1947). In all experiments plerocercoids from *Salmo trutta* were used.

2. *Diphyllobothrium ditremum* (Creplin, 1825), redescribed by Matz (1891), was obtained experimentally by Hickey and Harris (1947) from *Phalacrocorax aristotelis* fed with plerocercoids from *Salmo trutta*. These authors also found this parasite naturally in *Phalacrocorax aristotelis* and *P. carbo*. Attempts to infect gulls apparently failed.

3. *Diphyllobothrium fissiceps* (Creplin, 1829) was originally described from *Sterna hirundo*, and does not appear to have been met with again.

4. *Diphyllobothrium cordiceps* (Leidy, 1872) was originally described as a plerocercoid in the body-cavity of *Salmo mykiss*. Later, Linton (1891), basing his observations on the description given by Leidy, describes the adult form as a parasite in the intestine of *Pelecanus erythrorhynchus*. Simms and Shaw (1931) obtained this tapeworm by feeding to *Larus occidentalis* plerocercoids from *Salvelinus fontinalis* and *Oncorhynchus kisutch*. None of these authors gives any details of the genital organs.* Woodbury (1935) tried to infect himself by swallowing plerocercoids, which he considered to be young stages of *D. cordiceps*, from *Salmo lewisii*, but without success.

5. *Diphyllobothrium exile* (Linton, 1892). The original description of this species was based on young immature specimens found in *Larus californicus*.

6. *Diphyllobothrium canadense* Cooper, 1921, was originally found in *Corvus principalis*.

7. *Diphyllobothrium* sp. Markowski, 1933. This indeterminate form consisted of immature specimens from *Sterna hirundo*.

* Skinker (1932) considers *D. cordiceps* as a synonym of *Diphyllobothrium latum*.

8. *Diphyllobothrium oblongatum* Thomas, 1946. This species was obtained experimentally by Thomas (1947) from *Larus argentatus* fed with plerocercoids from *Leucichthys sp.* Thomas found the final-host also to be naturally infested.†

In connection with the eight avian species mentioned above, the writer is disposed to mention two more species, namely, *Diphyllobothrium strictum* (Talysin, 1932) and *D. laruei* Vergeer, 1942. The first of these species was recorded as a parasite of man, while the second was obtained experimentally from a dog which had been fed with plerocercoids from *Leucichthys artedi*. Judging from their descriptions, it appears that both species are synonyms of *D. dendriticum*.

All details concerning the occurrence of plerocercoid larvae in Salmonoid fishes and of adult stages in natural and experimental hosts, as well as their geographical distribution, have been enumerated in Tables II and III.

Original Observations.

The lack of comparability in the descriptions available for most of the species under consideration has been the cause of much confusion regarding the identity of these species. To simplify the problem of identification, the writer has paid special attention to those anatomical features which are of real systematic value, easy to discern, and not subject to great variation.

The features which may be regarded as specific criteria are :—(1) presence or absence of a "neck"; (2) the shape of the segments throughout the strobila; (3) the number of testes, their shape, size and disposition, and their relation to other organs of the genital complex; (4) the maximum number of testes in sagittal and transverse sections; (5) the number of uterine loops on each side of the segment; (6) the modifications of the uterus occurring in the hinder portion of the strobila; (7) the relation of the uterine loops to the cirrus-sac; (8) the shape of the ovary; and (9) the relation of the cirrus-sac to the anterior border of the segment. These criteria are introduced into the descriptions given below of the writer's material of *Diphyllobothrium dendriticum* and *D. ditremum*.

† A cestode, possibly a species of *Diphyllobothrium*, was inadequately described by Rennie and Reid (1912) as *Dibothriocephalus pygoscelis* from a penguin (*Pygoscelis* sp.). It appears, however, that the material "was found . . .lying on the snow near the beach at Scotia Bay, South Orkneys, where a number of penguins had been congregated." This evidence is not sufficiently conclusive to allow the penguin to be regarded as the true host of this species. *D. pygoscelis* is, therefore, not considered in the present work.

Both species have been redescribed by Matz (1891), who unfortunately had at his disposal a very limited amount of material, consisting of one headless specimen of *D. dendriticum* and three specimens of *D. ditremum*. He is the first author to give the number of testes in transverse and sagittal sections of *D. ditremum*. The data given by Matz (1891) have been repeated by Luehe (1910), who, probably by a *lapsus*, gives the length of the body in *D. dendriticum* as 13 mm. instead of 130 mm. Having a considerable amount of material from aquatic birds, the writer has been able to add further details to the descriptions given by earlier authors. Finally, the differences between the two species observed in this material, and their hosts, have been enumerated in Table I.

Diphyllobothrium dendriticum (Nitzsch, 1824).
(Figs. 1–6.)

Synonymy : *Bothriocephalus dendriticus* Nitzsch, 1824 ; *Bothriocephalus fissiceps* Creplin, 1829 ; *Bothriocephalus cordiceps* of Braun, 1894 ; *Dibothrium dendriticum* of Diesing, 1850 ; *Dibothrium fissiceps* of Diesing, 1850 ; *Dibothrium cordiceps* Leidy, 1872 ; *Dibothrium exile* Linton, 1892 ; *Bothriotaenia fissiceps* of Ariola, 1896 ; *Dibothriocephalus dendriticus* of Luehe, 1899 ; *Dibothriocephalus strictus* Talysin, 1932 ; *Diphyllobothrium dendriticum* of Luehe, 1910 ; *Diphyllobothrium fissiceps* of Luehe, 1910 ; *Diphyllobothrium canadense* Cooper, 1921 ; *Diphyllobothrium cordiceps* of Meggitt, 1924 ; *Diphyllobothrium exile* of Meggitt, 1924 ; *Diphyllobothrium* sp. innom. Markowski, 1933 ; *Diphyllobothrium strictum* of Neveu-Lemaire, 1936 ; *Diphyllobothrium laruei* Vergeer, 1942 ; *Diphyllobothrium oblongatum* Thomas, 1946.

Material which is assigned to this species has been examined from the following hosts :—*Larus marinus*, *L. argentatus*, *L. fuscus*, *L. californicus*, *L. delawarensis*, *Pelecanus erythrorhynchus*. *L. delawarensis* is recorded for the first time as a host for this species.

The body is whitish, brownish or grey. In the fully mature, elongate segments the lateral areas, owing to the presence of vitelline glands, are darker in colour than the middle region. The dimensions of the strobila are extremely variable, but in the present specimens the maximum length is about 42 cm. and the maximum width about 6 mm.

In different parts of the strobila the segments differ considerably in shape. In the anterior half they are somewhat trapezoid, the length being much less than the width. This shape later becomes oblong, then elongate, and finally the older segments become somewhat thread-like. The latter segments are degenerate and contain a few scattered deformed eggs. The lateral margins of the fully gravid segments are

concave, which causes the anterior and posterior edges to appear prominent. In the anterior half of the strobila the segments measure up to 3 mm. in length and 6 mm. in width, and in the posterior half the fully gravid elongate segments measure up to 6 mm. and 2 mm. respectively. The scolex measures 1.2–2 mm. in length and about 0.6 mm. in dorso-ventral width and is more elongate than that of *D. ditremum*. The " neck " is readily distinguished from the remainder of the strobila, measures 2–4 mm. in length and averages about 0.4 mm. in width. The longitudinal muscles of the body are very well developed (much more so than in *D. ditremum*) and are conspicuous in transverse sections as a deep layer.* The genital organs are disposed in one to four sets arranged tandem in each segment. When only one set occurs it is situated near the centre of the segment. Where two or more sets occur, the best developed of them lies very near the posterior border of the segment and contains most eggs. The other sets are in different stages of maturity and contain fewer eggs, and sometimes may consist only of the cirrus-sac.

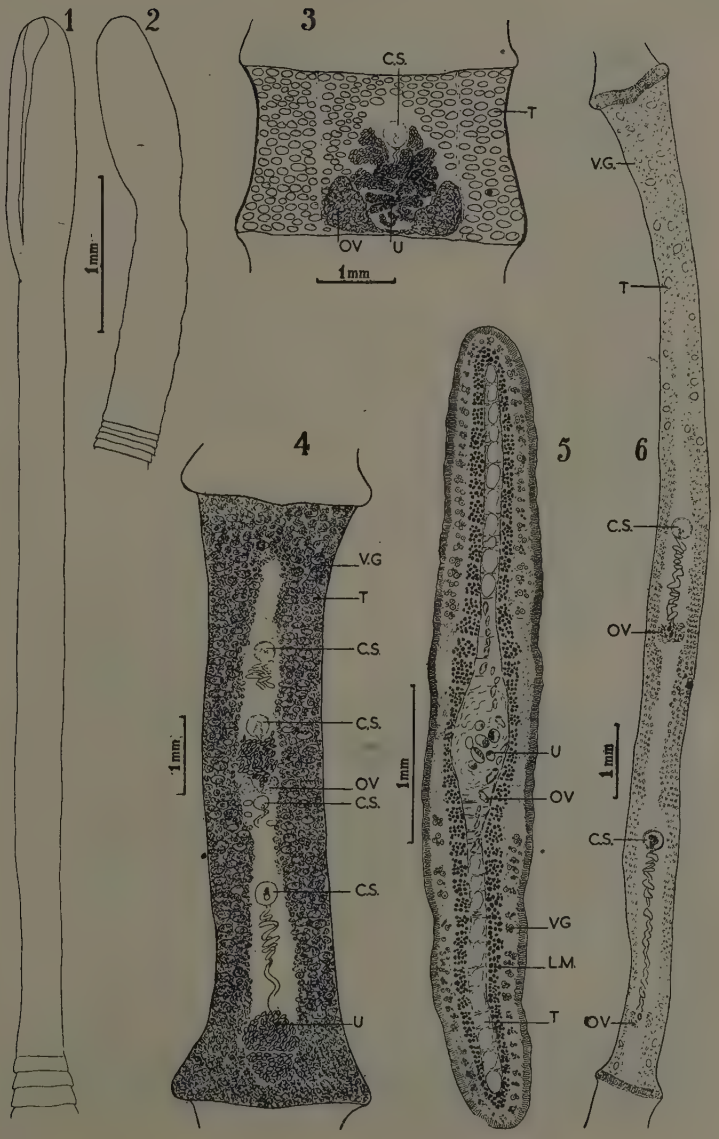
The cirrus-sac and the uterus are surrounded by an elongate-oval translucent area, which is typical of *D. dendriticum*. This area is entirely free of vitelline glands.

The testes are disposed in a single layer in the medullary parenchyme. They occupy the lateral fields and are confluent in the anterior region of each segment. They are less densely arranged than in *D. ditremum* and are not contiguous with the uterine loops. The shape of the testes is irregularly spherical or somewhat ovoid. In the latter case the longer axis of each is usually transverse to the main axis of the body. They measure 99–165μ × 62–148μ. The number of testes in a segment varies from 168 to 408, and this variation may be observed in a single specimen. The maximum number of testes to be seen in sagittal sections between the lateral margin of the segment and the main excretory vessel and nerve cord is 38. In transverse sections

* In *D. latum* the longitudinal muscles are collected into more distinct bundles and differ considerably from those of *D. dendtriticum* and *D. ditremum*.

Diphyllobothrium dendriticum.

Fig. 1. From *Larus marinus*. Fully extended scolex; lateral view. Fig. 2. From Kitten. Scolex; dorso-ventral view. Fig. 3. From *Larus marinus*. Gravid segment (vitelline glands omitted, arrangement of testes diagrammatic). Fig. 4. From *Larus marinus*. Fully gravid segment with four sets of genital organs. Fig. 5. From *Larus fuscus*. Transverse section in the region of the ovary, showing the arrangement and development of the muscles. Fig. 6. From *Larus marinus*. Thread-like spent segment with remnants of eggs. (OV. ovary; T. testes; U. uterus; V.G. vitelline glands; C.S. cirrus-sac; L.M. longitudinal muscles).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the maximum number occurs in the anterior region of the segment and amounts to 30. Towards the centre of the segment, in both sagittal and transverse planes, the number of testes decreases.†

The cirrus-sac measures approximately 300 × 300μ. It is situated some distance from the anterior border of the segment, whereas in *D. ditremum* the cirrus-sac lies near the anterior border. Only in some of the squarish segments of *D. dendriticum* does the cirrus-sac lie relatively nearer to the anterior border. The cirrus-sac is situated anteriorly to the uterus, and the first pair of uterine loops may extend to its middle, but it is seldom surrounded or embedded in these loops, as is the case in fully gravid segments of *D. ditremum*.

The ovary is, in its early stages, bilobed, later becoming bean-shaped. It has a vesicular structure and possesses a transverse isthmus of a similar nature. The vitelline glands are situated in the dorsal and ventral regions of the cortical parenchyme, and are confluent in the median line of each segment anteriorly and posteriorly. The follicles measure 66 × 66μ to 66 × 85μ. The uterus consists of 9 to 11 loops on each side. The golden-yellow, operculated eggs measure about 56–60μ × 40μ. In the hinder portion of the uterus the eggs have a blackish, transparent appearance. At the opposite end to the operculum, the egg-shell is sometimes provided with a small boss.

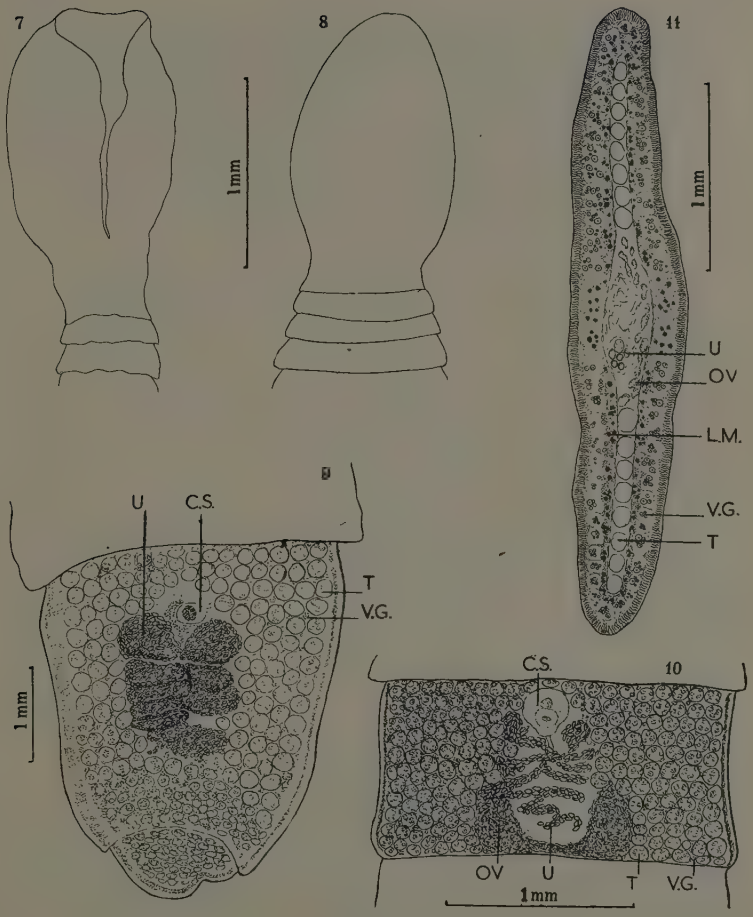
Diphyllobothrium ditremum (Creplin, 1825).
(Figs. 7–11.)

Synonymy: *Bothriocephalus ditremus* Creplin, 1825; *Dibothrium ditremum* of Diesing, 1850; *Dibothriocephalus ditremus* of Luehe, 1899; *Diphyllobothrium ditremum* of Luehe, 1910.

The material upon which the writer's original observations are based was obtained from the following hosts:—*Phalacrocorax carbo, P. aristotelis, Mergus americanus, Ardea cinerea. M. americanus* is recorded for the first time as a host for this species.

The dimensions of the strobila are variable. The maximum length obtained from the writer's specimens is 14 cm. and the maximum width 2.5 mm. Individuals measuring 4 cm. in length are fully developed and possess gravid segments. The shape of the segments is

† In comparing *D. dendriticum* with a specimen of *D. latum*, the host of which was probably a dog, some differences have been observed. In the latter species, the maximum number of testes in sagittal section is 35; in transverse section 18, which seems to occur behind the cirrus-sac. The segment from which these figures were obtained measured 5.5 mm. in length and 3 mm. in width and contained fully-developed female organs, with eggs.



Diphyllobothrium ditremum.

Fig. 7. From *Phalacrocorax carbo*. Scolex; lateral view. Fig. 8. From *Phalacrocorax carbo*. Scolex; dorso-ventral view. Fig. 9. From *Phalacrocorax aristotelis*. Terminal segment. Fig. 10. From *Phalacrocorax carbo*. Mature segment. Fig. 11. From *Phalacrocorax carbo*. Transverse section in the region of the ovary, showing the arrangement and development of the muscles. (OV. ovary; T. testes; U. Uterus; V.G. vitelline glands; C.S. cirrus-sac; L.M. longitudinal muscles).

trapezoid, or more or less square, with a well-developed posterior margin, which is rounded in the last segment. The scolex measures 1.5 mm. in length and about 1 mm. in dorso-ventral width and is more oval in shape than that of *D. dendriticum*. Segmentation begins immediately behind the "head." The longitudinal muscles of the body are not so well developed as they are in *D. dendriticum*, and form a shallower layer in transverse section. A translucent area does not occur around the cirrus-sac and uterus in this species.

TABLE I.

Specific differences between *Diphyllobothrium dendriticum* and *D. ditremum*, based on new material.

Species	*D. dendriticum*	*D. ditremum*
Body : length	42 cm.	14 cm.
Body : width	6 mm.	2.5 mm.
Scolex : length	1.2–2 mm.	1.5 mm.
Scolex : width	500μ–600μ	975μ
Neck : length	2–4 mm.	Neck absent.
Neck : width	390μ	
Segment : length	3–6 mm.	3 mm.
Segment : width	5–2 mm.	2.5 mm.
Shape of segment :	Trapezoid, squarish or several times longer than wide.	Trapezoid or squarish.
Testes, No. per segment :	168–408	190–380
Diameter of testes :	99μ–165μ × 62μ–148μ	180μ × 165μ–150μ × 275μ
Shape of testes :	Rather ovoid with larger axis transverse to main axis of strobila.	Rather irregularly spherical or ovoid.
Maximum No. of testes in sagittal section of segment :	38	13
Maximum No. of testes in transverse section of segment :	30	16–20
Cirrus-sac, diameter :	300μ × 300μ	270μ × 300μ
Position of cirrus-sac in segment :	Near centre. In early mature segment near anterior border.	Near anterior border.
Relation of cirrus-sac to uterine loops.	First uterine loops may reach middle of cirrus-sac.	First uterine loops surround cirrus-sac or reaching its middle.
No. of sets of genital organs in segment :	1–4	1
Shape of fully-developed ovary :	Bean-shaped.	Rather crescent-shaped.
No. of uterine loops on each side of segment :	9–11	4–6
Eggs, size :	56μ–59.4μ × 39.6μ	53μ–56μ × 36μ–40μ
Vitelline glands, diameter :	66μ × 66μ–85μ	72.6μ–89μ × 76μ–99μ

The testes are situated in the medullary parenchyme. They are disposed in a single layer on each side of the median line, confluent anteriorly, and extending medially as far as the walls of the cirrus-sac

and uterine loops. Their number varies between 190 and 380. In sagittal section, between the lateral margin of the segment and the main excretory vessel, they number up to 13, and in transverse section of the anterior portion of the segment from 16 to 20. They measure $180 \times 165\,\mu$—$150 \times 275\mu$. The cirrus-sac is more or less spherical and measures $270 \times 300\mu$ in diameter. It is situated very close to the anterior border of the segment. In young segments, where the eggs are not numerous, the cirrus-sac is anterior to the uterine coils. Gradually, however, in older segments the coils envelop the cirrus-sac and extend as far as its anterior border.

The ovary is rather crescent-shaped and situated on the posterior margin of the segment. The vitelline glands are situated in the cortical parenchyme and reach to the anterior border of the segment. The follicles are irregularly spherical and measure 72—$89\mu \times 76$—99μ. The uterus forms 4 to 6 loops on each side of the segment and contains 200–300 eggs. These loops are loosely arranged in a few of the younger segments. In older segments, however, the loops become swollen with masses of eggs, and gradually the uterus loses its looped appearance. Finally, in very old gravid segments, the uterus is converted into an irregularly spherical sac. The anterior region of the modified uterus possesses a V-shaped cavity, in which lies the cirrus-sac. The golden-brown, operculated eggs measure 53–$56\mu \times 36$–40μ. In the lower portion of the uterus the eggs have a blackish, transparent appearance. The egg-shell is sometimes provided with a boss at the end opposite to the operculum. This boss is slightly larger than that in the eggs of *D. dendriticum* and occurs more frequently.

Critical Remarks.

The descriptions of individual species of *Diphyllobothrium* given by early writers are very inadequate and often difficult to compare. This may be seen in Tables II and III, which have been compiled from data available in the literature. The early writers distinguished their species mainly by such characters as the dimensions of the strobila, number of segments, size of scolex and of " neck." These features are so variable that they can scarcely be considered as specific criteria.

According to the writer's observations, specimens of *Diphyllobothrium ditremum* measuring 4 cm. and 14 cm. in length have each reached a similar degree of sexual maturity, inasmuch as the uterine coils contain eggs. A similar relationship between size and sexual maturity may be observed in *D. dendriticum*.

The " neck " is of systematic value only in so far as it is present or

TABLE II.

Comparison of morphological data recorded by earlier authors for *Diphyllobothrium* species from birds and other hosts.

	Species	*dendriticum*	*ditremum*	*fissiceps*	*cordiceps*	*exile*	*canadense*	*strictum*	sp. indet.	*laruei*	*oblongatum*
Body :	length	42.5 cm.	12.9 cm.	15 cm.	75 cm.	15.3 cm.	23.5 cm.	47 cm.	7 mm.	11.2 cm.	57 cm.
	width	7.25 mm.	2.33 mm.	5 mm.	7.5 mm.	0.8 mm.	6.5 mm.	4 mm.	1 mm.	2.103 mm.	2 mm.
Scolex :	length	1.15 mm.	1.16 mm.	elongate	2 mm.	1.5 mm.	0.84 mm.	?	?	1.667 mm.	1.75 mm.
	width	0.62 mm.	1 mm.	?	0.75 mm.	0.6 mm.	0.46 mm.	?	?	0.859 mm.	0.44 mm.
Segment :	max. length	6 mm.	2.25 mm.	length four	4 mm.	0.85 mm.	0.65 mm.	7 mm.	?	length three	7 mm.
	max. width	2 mm.	2.33 mm.	times width	7.5 mm.	0.80 mm.	3.5 mm.	2 mm.	?	times width	2 mm.
Neck :	length	1.5 mm.	neck absent	neck absent	2–6 mm.	long & slender	0.7 mm.	?	?	1.59 mm.	1.09 mm.
	width	?			0.75 mm.	0.3 mm.	?	?	?	0.333 mm.	0.7 mm.
Testes, No. per segment		470	380–390	?	?	?	150	?	?	225–400	264–318
Diameter of testes		94.5μ	101μ	?	?	?	55–90μ ×110–145μ	?	?	65μ–81μ	87μ×131μ
Cirrus sac :	antero-posterior diam.	409μ	282μ	?	?	?	320μ	?	?	142μ–164μ	175μ–201μ
	transverse diam.	327μ	164μ	?	?	?	200μ	?	?	?	306μ
Shape of ovary		?	?	?	?	?	irregularly lobed laterally	bean-shaped	?	bean-shaped	bean-shaped
No. of uterine loops on each side of segment		8–9	7	?	?	?	8–10	?	?	7–8	7–8
Eggs, size		?	?	?	?	?	56–59μ×37–39μ	54–64μ ×40–44μ	?	41–52μ×30–37μ	61μ×39μ
Vitelline glands, size		?	?	?	?	?	40μ×75μ	?	?	35–46μ×32–46μ	15μ×16μ

absent, because the length of this part of the body varies considerably, even in the same species, and is in no way constant. Variations in the length of this region of the body are often due to contraction, and may be caused by fixatives.

TABLE III.

Further data concerning *Diphyllobothrium* spp. in Table II.

Species	Definitive hosts	Second intermediate hosts	Geographical distribution	Authors
dendriticum.	*Larus ridibundus,* *L. canus,* *L. argentatus,* *L. fuscus,* *Rissa tridactyla.* Experimental : Dog, cat, rat. *Larus marinus,* *L. argentatus.*	*Salmo trutta.*	England. Eire. Germany.	Matz, 1891. Luehe, 1910. Duguid and Sheppard, 1944. Baylis, 1945. Hickey and Harris, 1947.
*ditremum.**	*Colymbus arcticus,* *C. stellatus,* *Mergus merganser,* *M. serrator,* *Phalacrocorax carbo,* *P. aristotelis,* *Ardea cinerea, Larus argentatus.* Experimental : *Phalacrocorax aristotelis.*	*Salmo trutta.*	England. Eire. Germany.	Matz, 1891. Luehe, 1910. Duguid and Sheppard, 1944. Hickey and Harris, 1947.
fissiceps.	*Sterna hirundo.*	?	Germany.	Luehe, 1910.
cordiceps.	*Pelecanus erythrorhynchus,* *Larus californicus.* Experimental : *Larus occidentalis.*	*Salvelinus fontinalis,* *Salmo mykiss,* *Salmo lewisi,* *Onchorhynchus kisutch* (in cysts).	Yellowstone Park. Elk River. Yellowstone River, Wyoming, U.S.A.	Leidy, 1872. Linton, 1891. Linton, 1892. Simms and Shaw, 1931.
exile.	*Larus californicus.*	?	Yellowstone Park Lake, Wyoming, U.S.A.	Linton, 1892.
canadense.	*Corvus principalis.*	?	Bernard Harbour, Canada.	Cooper, 1921.
strictum.	*Homo sapiens.*	?	Olchon Island, Baikal Lake.	Talysin, 1932.
sp. indet.	*Sterna hirundo.*	?	Hela, Poland (Baltic).	Markowski, 1933.
laruei.	Experimental : Dog.	*Leucichthys* sp. (in cysts).	Great Lakes of North America.	Vergeer, 1942.
oblongatum.	Natural and Experimental : *Larus argentatus.*	*Leucichthys artedi* (in cysts).	Great Lakes of North America.	Thomas, 1946.

*Linstow (1905) records *D. ditremum* from *Larus glaucus* at West Tajmyr, but it seems very likely that this material has been incorrectly determined. In a list of the hosts previously recorded for *D. ditremum*, Linstow includes *Mergus albellus*. This may have been an error for *Mergus merganser*, which is omitted from the list, but which is recorded as a host for *D. ditremum* by Linstow (1878).

The number of testes is also very unreliable as a systematic criterion because in the same species, and even in a single worm, the maximum number may be twice as great as the minimum. The dimensions of the testes vary according to the degree of development.

Some contemporary authors, *e.g.* Vergeer (1942) and Thomas (1946) introduce into their descriptions new features, among which are the measurements of the ootype, of the receptaculum seminis and of the genital apertures. These features are also of little systematic value, since they may undergo individual variation caused by the action of fixatives, as well as by the excessive development of the genital products. For this reason these features have not been included in Table II. Moreover, only two species are concerned, namely *D. laruei* and *D. oblongatum*, described by Vergeer and Thomas respectively.

After comparing the data obtained from the literature and recorded in Table II with those arrived at by the writer and presented in Table I, it will be seen at a glance that one species (*Diphyllobothrium ditremum*) may be readily separated from the others. Superficially, this species differs markedly from the remainder by the absence of a " neck." Furthermore, *D. ditremum* apparently occurs only in Phalacrocoracidae, Anatidae, Ardeidae and Colymbidae.* *D. ditremum* was obtained experimentally by Hickey and Harris (1947) from *Phalacrocorax aristotelis*, and attempts by these authors to infect gulls with this species proved unsuccessful. It can therefore be supposed that this parasite is specific to a limited number of host-species. It happens, however, that the plerocercoid stage of *D. ditremum* occurs in *Salmo trutta*, as does that of *D. dendriticum*.

In spite of the confusion caused by the incompleteness of the descriptions published during the last century, it is possible to note some features which are common to the remaining nine species mentioned above. These features are :—(1) a " neck " is present ;† (2) very narrow elongate segments, becoming somewhat threadlike, occur in the posterior region of the body ; (3) in its early stages of development the ovary is bilobed, later becoming bean-shaped ; (4) the segments may sometimes contain one to four sets of genital organs ; (5) the adult worms occur in Corvidae, Pelecanidae and Laridae ; (6) the worms possess the facility to adapt themselves to and reach sexual maturity in final hosts that are unrelated from a physiological and systematic standpoint (*e.g.*, bird, cat, dog, rat and man). All these features are associated with *D. dendriticum*.

* Luehe (1910) mentions that *D. ditremum* has once been recorded from *Larus argentatus*, but this seems to be very doubtful.

† The possible exception is to be found in *D. fissiceps*, in which a " neck " has not been described.

Notwithstanding the inconsistent and inadequate descriptions available for the older species, an attempt has been made to show that with the exception of *D. ditremum* the species listed in Tables II and III are comparable. In *D. fissiceps*, for example, the segments are four times longer than broad, a feature agreeing entirely with *D. dendriticum*. No details were given by Creplin (1829) regarding the genital glands. The apparent absence of a " neck " might have been caused by contraction. The host, *Sterna hirundo*, is a representative of the Laridae, which harbour *D. dendriticum*.

The next two species, *D. cordiceps* and *D. exile*, agree with *D. dendriticum*, since their segments are elongate. Very old segments of *D. cordiceps* become threadlike, a feature found in the writer's material of *D. dendriticum*. In both *D. cordiceps* and *D. exile* the " neck " is well developed. Unfortunately, the description of *D. cordiceps* contains no account of the genital organs, and that of *D. exile* is based entirely on specimens in which genital organs had not yet developed.

A comparison of some specimens received from Dr. Price, labelled " *D. cordiceps* " from *Pelecanus erythrorhynchus*, with material from European and American gulls, shows that they are identical with the latter. Both *D. cordiceps* and *D. exile* have been recorded from *Larus californicus* and they may be considered as synonyms of *D. dendriticum*.

One of Cooper's (1921) drawings of *D. canadense* appears to have been taken from a trapezoid-like segment, which is mature and producing eggs. Segments of this shape are to be found in the anterior half of the body of *D. dendriticum*. It must be added, however, that segments of a similar shape are to be found also in *D. ditremum*, and it appears very probable that Cooper's material did not include any of the elongate segments that usually occur in fully adult specimens of *D. dendriticum*. The bilobed ovary depicted by Cooper is similar in shape to that occurring in the early mature segments of *D. dendriticum*, in the older segments of which the ovary becomes bean-shaped. The arrangement of the testes shown in Cooper's drawing is very characteristic of *D. dendriticum*. The testes are well separated from other organs of the genital complex, and their longer axes are transverse to the main axis of the body. Cooper estimates their number to be 150, and this figure closely agrees with that given for *D. dendriticum* (see Table I). A similar relationship has been observed in the position of the cirrus-sac and in the number of the uterine loops in both species. It can be supposed that, under the name of *D. canadense*, Cooper was dealing with material of *D. dendriticum*, in which the strobila had not yet reached that stage of development where the segments become

elongate.

The young specimens of an indeterminate species of *Diphyllobothrium* recorded by the writer (1933) from *Sterna hirundo* may be considered as an early intestinal stage of *D. dendriticum*, because the host in which they were found belongs to the Laridae, the typical hosts of the latter species. No genital rudiments were observed in the young specimens.

A comparison of the description of *D. strictum*, recorded by Talysin (1932) from man, with *D. dendriticum* shows that they are very similar, in spite of the inadequate description given for the former. Both have almost the same length of strobila (see Tables I and II), and very elongate segments, which may sometimes contain double sets of genital organs arranged tandem. Talysin does not give the number or the size of the testes, nor the number of uterine coils. He mentions that the colour of the strobila is light brown, and the writer has seen similarly coloured specimens of *D. dendriticum* obtained from *Larus fuscus*. The similarity of the common features in both species allows the supposition that *D. strictum* is a synonym of *D. dendriticum*.

The experimental work carried out by Duguid and Sheppard (1944) and by Hickey and Harris (1947) shows that the dog, cat and rat are susceptible to infection with *D. dendriticum*. Hence it is not improbable that man may also serve as a host. Although the attempt of Woodbury (1935) to infect himself was unsuccessful, the experiment is inconclusive, because in some of the experimental hosts used by the previously mentioned authors, adult worms did not always develop. Further, it is possible that Woodbury used for his experiment the plerocercoid of another species of Pseudophyllidean, because, as is well known, this type of larva is often impossible to identify specifically. According to Talysin, the population of Olchon Island, where he found *D. strictum*, lives under very primitive hygienic conditions, using for food insufficiently cooked or salted, or even raw fish. It may be assumed that the people eat, among others, Salmonoid fishes, and in this way become infected with this species of parasite. Since, therefore, *D. strictum* may be considered as a synonym of *D. dendriticum*, it is possible that in Europe infection may occur as a result of swallowing plerocercoids from *Salmo trutta*. Thus, from a medical point of view, *D. dendriticum* may be of some importance as a human parasite in very specific and favourable conditions.

D. laruei, described by Vergeer (1942), was obtained experimentally in a dog which had been fed with plerocercoids from *Leucichthys artedi*. It resembles *D. dendriticum* in the elongate shape of the gravid segments, in the size of the testes and their number, in the bean-shaped ovary,

and in the number of uterine coils. The features shown in a drawing of a segment given by Vergeer are identical with those found in some of the present material from *Larus fuscus* and *L. argentatus*.

Thomas (1946) obtained his species, *D. oblongatum*, from naturally and experimentally infested *Larus argentatus* which were, for the experimental work, fed with plerocercoids from *Leucichthys* sp. The specimen of *D. oblongatum* received from Professor Thomas resembles the writer's material from *L. argentatus* in the shape of the narrow, elongate gravid segments, in the presence of a double or triple set of genital organs in a single segment, in the number and size of the testes, and in the number of uterine coils. The shape of the ovary agrees with that in *D. dendriticum*. The figures given by Thomas of a single segment and of the entire strobila agree also with the general appearance of the present specimens of *D. dendriticum*.

The plerocercoids used by Vergeer (1942) and Thomas (1946) were from the same locality, the Great Lakes of North America. The results of the experiments carried out by these authors are very similar to those obtained by Hickey and Harris (1947). Vergeer and Thomas each used plerocercoids from the same genus of fish, *Leucichthys*, and obtained adult stages in the dog and in *Larus argentatus*. Hickey and Harris, on the other hand, using plerocercoids from *Salmo trutta*, obtained adult stages in the dog, in *Larus argentatus* and in *L. marinus*. It may be supposed that all these authors were dealing with the same species of tapeworm, namely *D. dendriticum*.

Thus, from a comparison of the characters of the species described by previous authors and those observed in the writer's material, it may be assumed that *D. fissiceps*, *D. cordiceps*, *D. exile*, *D. canadense*, *D. strictum*, *D. laruei*, *D. oblongatum*, and *D.* sp. Markowski are all synonyms of *D. dendriticum*.

To sum up, regarding the validity of the species of *Diphyllobothrium* from birds, it appears that there exist only two distinct species, namely, *D. dendriticum* and *D. ditremum*.

Summary.

A comparative study has been made of certain species of *Diphyllobothrium* which are primarily parasites of birds, but which have been obtained from man and other hosts, both naturally and experimentally. As a result of this work it appears that only two valid species are known in birds, namely: *Diphyllobothrium dendriticum* (Nitzsch, 1824) and *D. ditremum* (Creplin, 1825). These species differ markedly from each other in certain morphological features and there appears to be a notice-

able difference in their host-adaptation, inasmuch as *D. ditremum* occurs only in birds belonging to the families Phalacrocoracidae, Ardeidae, Colymbidae and Anatidae, while *D. dendriticum* occurs naturally in birds belonging to the Laridae and other families, and experimentally also in mammals.

It appears that *D. dendriticum* is capable of being a natural parasite of man, and therefore may be of medical importance in special circumstances.

Finally, *Mergus americanus* is recorded as a new host for *D. ditremum*, and *Larus delawarensis* for *D. dendriticum*.

References.

It is felt that a full bibliography of all the literature bearing on the occurence of *Diphyllobothrium* in birds would be of considerable value. In order to save space, however, only the more recent references are given in full, and for complete references to the following abbreviated citations the reader is referred to Stiles, C. W. and Hassall, A., 1902–1912, Index Catalogue of Medical and Veterinary Zoology (Authors).

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The Eggs of *Schistosoma bovis*, *S. mattheei* and *S. haematobium*.

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In 1929, Veglia and Leroux described a schistosome from sheep in the Cape Province of South Africa. Unfortunately, they were unable to obtain either type specimens of *S. bovis* or Sonsino's original description of the parasite, and were compelled to base their comparisons on material obtained from Khalil, and upon his description of the parasite as it occurs in cattle in Egypt. It is perhaps noteworthy in this connection, that Van den Berghe (1937) states that *S. bovis* in Egypt is an importation from the Sudan. Their lack of acquaintance with Sonsino's work led them into the erroneous belief that he had described *S. bovis* as a parasite of both the uro-genital and the intestinal tracts of cattle. Actually, Sonsino makes no mention of uro-genital infestation. Having concluded that they were dealing with a new schistosome, they named it *S. mattheei* after Mr. Matthee, the farmer whose sheep were harbouring the parasite. Leroux (personal communication) afterwards found it frequently in cattle and sheep both in South Africa and in Northern Rhodesia.

Little attention had been paid to the potential infectivity of the animal schistosomes for man until Blackie (1932) described adults and ova, which he believed to be those of *S. mattheei*, and which he had recovered from man in Southern Rhodesia. He named cattle and baboons as natural hosts in the country. He found the ova in urine from African patients who were also passing eggs apparently indistinguishable from those of *S. haematobium*. In one case where eggs similar to those of *S. mattheei* were found in faeces of a human patient, Blackie elicited a history of eating half-raw ox-gut a little time before, and suspected that the eggs were from the ox-gut rather than from true parasites.

This suggestion that a parasite of stock could infect man was vigorously repudiated by McHattie, Mills and Chadwick (1933). Previously, McHattie and Chadwick (1932) had just as trenchantly attacked the validity of Veglia's and Leroux's new species. They

came to the conclusion—based on material from a wide area of Iraq, that Khalil's description of *S. bovis*—on which Veglia and Leroux had relied—required modification, and their new description of *S. bovis* showed that *S. mattheei* could not be differentiated from it. They showed also that *in utero*, eggs of *S. bovis* are remarkably polymorphic, ranging from the rounded appearance of *S. haematobium*, through the " lozenge " shape of *S. mattheei* to the typical *S. bovis* egg. They then used this polymorphism of *in utero S. bovis* eggs to attack Blackie's suggestion that man could be infected in Southern Rhodesia with *S. mattheei*.

In the writer's opinion it is distinctly illogical to argue, as they do, that since short, rounded eggs are the products of a " diseased " uterus or faulty shell glands in *S. bovis*, then long spindle-shaped eggs must be the products of diseased *S. haematobium*. Nor does it seem good logic to begin by denying the existence of a species—*S. mattheei*—and to proceed then to argue by analogy with *S. bovis* that this non-existent worm cannot infect man. No one has yet described the production of long spindle-shaped eggs by typical *S. haematobium*, and it remains a hypothesis. Nor does any discussion of diseased uteri and abnormal eggs, nor denial of the species existence, satisfactorily explain away Blackie's demonstration at human autopsies of adult worms which were neither *S. haematobium* nor *S. mansoni*.

Clayton Lane (1936) was content to sum up the evidence and declare against *S. mattheei* as a separate species, while Van den Berghe (1937) after some rather non-committal discussion suggested that *S. mattheei*, while indistinguishable from the *S. bovis* described by McHattie and Chadwick, should properly be called *S. bovis* var. *mattheei*. Although he appeared to agree with McHattie and Chadwick, he made the interesting statement that he can distinguish *S. mattheei* from *S. bovis* by microscopic examination. Leiper (1915) stated that *S. bovis* and *S. haematobium* adults were extremely difficult to differentiate when reared in a abnormal hosts. He also suggests that the larval forms of the mammalian schistosomes are so little different that it is unsafe to attempt to identify them by morphological means, and the only reliable method for identification is to infect laboratory animals and examine the adults thus obtained. Alves (1948) has already noted that *S. haematobium* cannot be reared to full maturity in the small laboratory animals normally employed, but examination of many thousands of cercariae from snails (*Physopsis* sp.) has failed to show any reliable morphological difference in them, although *S. mattheei*, *S. bovis* and immature *S. haematobium* have been reared from such

similar cercariae at different times.

The position is then, that we have three schistosomes, whose larval forms are up to now not differentiated, and which all produce terminal-spined eggs. The adults of the three worms are also very similar. The demonstration (Alves, 1948) of a female schistosome found *in copula* with what appeared to be a typical male *S. haematobium* at an autopsy on a native patient in Southern Rhodesia, obscures rather than clarifies this question of the morphology of the females of these three parasites. This specimen showed an equatorial ovary, vitellaria extending almost to the midline, and *haematobium*-like eggs in the uterus.

It cannot be questioned that eggs seen in the uteri of *S. mattheei* and *S. bovis* are remarkable for their polymorphism, and any attempt at differentiation of the species by examination of such uterine eggs would be exceedingly hazardous, in fact foolish. Although polymorphism is a feature of uterine eggs, it is unusual to find gross deviation in the size and shape of mature miracidium-containing eggs passed in the excreta of individually infected animals. In such examples one is impressed by the " spindle " shape of *S. bovis* eggs when they are compared with those of *S. mattheei*, and again there appears to be a definite difference between *S. mattheei* eggs and the *S. haematobium* eggs seen in human urine. Hitherto measurements of eggs have taken into account only two features, the length and maximum breadth, measurements which ignore the " spindle " character of *S. bovis*. In an attempt to give this " spindle " its true value, a third measurement has been introduced. Since the spindle characteristic is less marked in the non-spiked end of *S. mattheei*, and is to all intents and purposes lost in *S. haematobium*, an arbitrary distance of 50 microns from the non-spiked end was chosen and the breadth of the egg at that point was measured. It was hoped that the use of this third measurement would serve to separate all three species. Measurements were taken with a stage micrometer and a camera lucida, the eggs being drawn and afterwards measured with a rod calibrated against a standard magnification and micrometer. The material examined was goat, sheep and cattle faeces for *S. bovis*; mice, guinea-pig and hamster faeces for *S. mattheei*; and urine from West Africa, the Sudan and Southern Rhodesia for *S. haematobium* eggs. All the eggs measured contained miracidia. In the writer's opinion the presence or absence of a miracidium is more important in the production of polymorphism than the hypothetical disease of the worm. Five hundred eggs of each species were measured, it being considered that statistically valid deductions could be drawn from such a number.

Before discussing the statistical treatment of the figures obtained, it will be of interest to show some measurements. The new third measurement is given last in each case, the figures being summarised in Table I. All measurements are in microns.

TABLE I.

Summary of measurements of eggs.

Egg	*S. bovis*	*S. mattheei*	*S. haematobium*
Longest	232 x 71 x 43	232 x 76 x 56	170 x 75 x 65
Shortest	179 x 49 x 27	180 x 68 x 50	115 x 55 x 54
Broadest	222 x 73 x 42	212 x 82 x 65	132 x 80 x 74
Narrowest	194 x 40 x 24	182 x 49 x 33	130 x 46 x 44
Average	208 x 55 x 31	200 x 64 x 50	142 x 59 x 54

TABLE II.

Table of approximate probabilities.

Species	*S. bovis*	*S. mattheei*	*S. haematobium*	*Total*
S. bovis	96.9%	3.1%	0.0%	100%
S. mattheei	1.6%	98.4%	0.0%	100%
S. haematobium	0.0%	0.4%	99.6%	100%

These figures are not greatly important in themselves, but it is permissible to say that they at least show a very definite line of cleavage between *S. bovis* and *S. mattheei* on one hand and *S. haematobium* on the other. They show, too, that it would be difficult if not impossible to separate *S. bovis* and *S. mattheei* using the common length by breadth criteria, the first two figures in each case representing these features.

The method used to achieve a differentiation between the three species was based on the discriminant function analysis of Fisher as modified by Brown, and afterwards discussed and used by Rao (1947). Using this technique, it was possible to arrive at a graph on which the positions of each species could be plotted, by employing two sets of formulae to determine " X_1" and " X_2", the axes of the graph.

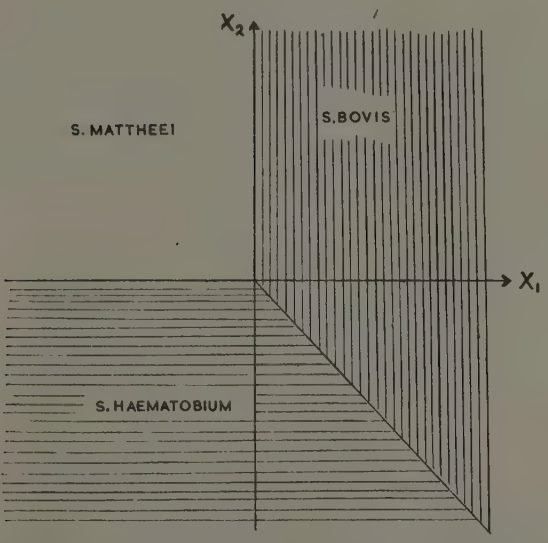

Fig. 1. Diagram for the discriminant functions X_1 and X_2, showing the regions into which the points corresponding to eggs of each species should fall.

The formulae are $X_1 = 0.46514x + 0.30226y - 1.88131z - 88.839$, and $X_2 = 1.08425x + 0.11858y - 1.427092z - 117.886$, when x = length, y = maximum breadth and z = breadth measured 50μ from the " blunt end " of the egg.

The diagram (Fig. 1) shows the regions into which the three species fall.

It is also possible by this method to calculate a table of approximate probabilities of correct or incorrect classification of the eggs using the formulae for X_1 and X_2 given above. It will be seen from Table II, which is largely self-explanatory, that normal *S. haematobium* and *S. bovis* eggs should never be wrongly classified as belonging to the other species. There is a three per cent. chance of wrongly classifying *S. bovis* as *S. mattheei*, and a one and one half per cent. chance of classifying *S. mattheei* as *S. bovis*. While an egg of *S. mattheei* should not be regarded as that of *S. haematobium* there is a half per cent. chance of misclassifying a *S. haematobium* egg as a *S. mattheei*.

It is of interest to note that there appear to be two distinct strains of *S. haematobium* eggs in those examined. The West African and Sudanese eggs, when they are plotted on the diagram shown in Figure 1, tend to congregate near the *mattheei* position, while the Rhodesian material remains well separated both from them and from the *mattheei* eggs. The largest *haematobium* egg, for example, was from a Sudanese specimen.

The average values of X_1 and X_2 for the three species are,

	X_1	X_2
S. bovis ...	18.21	70.52
S. mattheei ...	—18.21	34.76
S. haematobium	—54.00	—34.76

Discussion.

The use of the third measurement is largely responsible for bringing out the difference between the eggs of *mattheei* and *bovis*, since it will be seen from Table I that they are very similar in maximum, minimum and average lengths, and cannot be separated on the orthodox breadth measurements. The third measurement brings out the rapid narrowing to a spindle shape of *S. bovis*, and the much less obvious narrowing of *S. mattheei*.

Both eggs are much longer than those of *S. haematobium*, so much so that this difference should be appreciable visually, and it should normally be unnecessary to perform any other calculations, particularly with the small strain of *haematobium* egg encountered in South Central Africa.

It therefore appears that even although the adult forms of *S. mattheei* and *S. bovis* are extremely difficult to differentiate, a method of differentiation between the eggs is possible. Whether such a difference

is sufficient to justify the status of *S. mattheei* as a separate species is a question which will probably receive different answers from morphologists and biologists.

Summary.

1. Measurements of 500 eggs respectively of *S. bovis*, *S. mattheei* and *S. haematobium* were made. These measurements included length, maximum breadth, and breadth at a point 50 microns from the non-spiked end of the egg. Statistical analysis of the results shows that it is possible to differentiate between the three groups of eggs.

2. Reasons are adduced for suggesting that the statement that *S. mattheei* cannot infect man is not well founded : the question is under active investigation by the author.

Acknowledgments.

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A Review of the Trematode Genus *Galactosomum*.

By Stephen Prudhoe

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The genus *Galactosomum* was erected by Looss (1899) for the reception of *Monostomum lacteum* Jägerskiöld, 1896, an immature trematode occurring in cysts in the dura mater of *Cottus scorpius* on the west coast of Sweden. Later (1908), Jägerskiöld recovered specimens which he regarded as young adults of *Galactosomum lacteum* from the intestine of cormorants (*Phalacrocorax carbo*). Odhner (1910) recognized the close affinity between this genus and *Microlistrum* Braun, 1901, and expressed the view that a thorough study of adult material of *G. lacteum*, which had not then been adequately described, would be likely to show that the genera were synonymous. Pratt (1911) was apparently convinced of their identity and redescribed the type-species of *Microlistrum* under the name of *Galactosomum cochleariforme* (Diesing).

With the exception of Witenberg (1929), all subsequent writers appear to have accepted the opinion of Pratt unreservedly. Witenberg, however, regards the genera as distinct, " because of differences in the arrangement of the genital glands." This author does not specify the differences, but in his key to the genera of the subfamily Heterophyinae the arrangement of the testes appears to provide the chief means of separating the two genera : in *Galactosomum* the testes are said to lie " obliquely " and in *Microlistrum* " one behind the other." To be more explicit, in *Galactosomum lacteum* the testes lie, according to Jägerskiöld, obliquely one behind the other in the median line of the body, while in *Microlistrum* they are strictly tandem in the same plane. From the present writer's examination of adult specimens of *G. lacteum* it appears that the testes, although usually arranged obliquely as described by Jägerskiöld in the metacercaria, may occasionally be disposed tandem, as in *Microlistrum*. The latter disposition in this species seems to depend to a considerable extent upon the condition of the specimens at the time of fixation, as well as upon the number of eggs in the uterus. In specimens fixed in a well-extended condition the testes may be arranged tandem, while in others, in which the uterus is crowded with eggs, the testes may be moved into a similar position by loops of the uterus being forced between the lateral margins of the testes and the intestinal caeca. Conversely, in a new species to be described

below, the testes, although usually tandem, may sometimes be disposed obliquely, this condition having apparently been brought about either by the contraction of the body or by the ascending and descending branches of the uterus, which pass between the testes, becoming distended with eggs.

In *Galactosomum lacteum* the excretory vesicle appears to be mainly responsible for the oblique disposition of the testes. From the hinder end of the body the vesicle extends between the testes to the ovarian complex and assumes a somewhat sigmoid outline. In immature specimens it is rather voluminous, but in mature specimens, owing to pressure exerted by the surrounding uterus becoming distended with eggs, the vesicle tends to collapse, and its influence on the disposition of the testes is therefore considerably lessened, and sometimes lost. On the other hand, in *Microlistrum* the excretory vesicle reaches only as far as the hinder margin of the posterior testis, and therefore it seems that only the contraction of the body or the swelling of the uterus alters the normal tandem arrangement in this genus.

It is not possible, however, to correlate the anterior extent of the excretory vesicle with the usual arrangement of the testes as a means of distinguishing these genera, because in *G. humbargari* Park, 1936, the testes are said to be arranged obliquely and the excretory vesicle extends only as far as the hinder margin of the posterior testis.

Upon the evidence given above it does not seem possible to differentiate *Galactosomum* from *Microlistrum* merely by the arrangement of the testes, nor can such an arbitrary feature as the extent of the excretory vesicle in relation to the testes alone be considered generically important in this instance. Consequently, as these genera are otherwise morphologically indistinguishable they are here accepted as synonymous.

Price (1932) considers *Cercarioides* Witenberg, 1929, also as a synonym of *Galactosomum*, and this opinion appears to be justified. Later (1934), Price further remarks that a comparison of his new species *Galactosomum johnsoni* and *G. darbyi* with *Stictodora sawakinensis* Looss, 1899, " shows such close relationships that it appears doubtful whether *Stictodora* should be retained as a valid genus." It is true that the two former species, as well as *G. humbargari* Park, show closer affinities with *Stictodora* than any of the other species of *Galactosomum*. However, there is as yet no conclusive evidence to show that these genera are identical, and, so far as existing descriptions of their species permit, their main differences have been enumerated in the following table :

	Galactosomum	*Stictodora*
Body :	Elongate, sometimes broadly spatulate anteriorly and cylindrical posteriorly.	More or less pyriform, tapering anteriorly, broadly rounded posteriorly.
Gonotyl :	Smooth, or with minute bristles or slender spines.	With strongly-curved pointed hooks.
Seminal vesicle :	Entire, or constricted into two elongate portions.	Constricted into three or four rounded portions.
Testes :	Closely approximate, one behind the other, directly or obliquely.	Well separated, side by side, directly or obliquely.
Ovary :	Distinctly anterior to foremost testis.	Level with anterior testis, or between testes.
Vitelline follicles :	Extending anteriorly beyond foremost testis.	Confined to area posterior to testes.
Excretory vesicle :	Tubular.	Y-shaped.

These differences appear to be constant and sufficiently characteristic to warrant, at least for the present, the retention of *Stictodora* as a distinct genus.

Lastly, Srivastava (1935) erects the genus *Tubanguia* for *Haplorchis anguillarum* Tubangui, 1933, but Tubangui and Africa (1938) assign this species to *Galactosomum* and there appears to be no justification for retaining *Tubanguia*.

The following diagnosis may now be given for the genus :—

Galactosomum Looss, 1899.

Synonymy : *Microlistrum* Braun, 1901 ; *Cercarioides* Witenberg, 1929 ; *Tubanguia* Srivastava, 1935.

Heterophyidae *sensu* Witenberg, 1929. Elongate forms in which the forebody is sometimes broadly spatulate and the hindbody more or less cylindrical. Cuticle beset with very small scale-like spines, which extend posteriorly to middle of body or beyond. Oral sucker simple, subterminal ; prepharynx relatively long ; pharynx well developed ; oesophagus short or absent. Excretory vesicle tubular, straight or somewhat sigmoid. Genital sinus spacious, complex, with a strongly muscular spiny pad attached to its right dorsal wall, and a gonotyl or tongue-like organ to its left wall. Seminal vesicle well developed, usually constricted into two portions. Testes rounded or somewhat oval, situated directly or obliquely one behind the other in posterior half of body. Ovary rounded or oval, median or lying to right of median line, anteriorly to testes. Receptaculum seminis large. Vitelline follicles lateral or intercaecal, confluent behind testes, extending anteriorly to foremost testis or beyond. Uterus descending and ascending between testes.

Adults parasitic in intestine of fish-eating birds ; metacercariae encysting in brain of marine fishes ; cercariae apparently not known. Two immature forms have been recorded from the stomach and intestine of a dolphin and an eel respectively.

Type-species : *G.* [*Monostomum*] *lacteum* (Jägerskiöld, 1896).

Owing to the lack of material it is not possible to bring within the scope of this paper a comprehensive revision of the morphology of all the species that have been assigned to this genus. However, an account of a new species and a description of adult specimens of the type-species are given, and the characteristics of the remaining species are briefly defined on the basis of information available in the literature.

1. *Galactosomum lacteum* (Jägerskiöld, 1896).
(Figs. 1 and 2)

Synonymy : *Monostomum lacteum* Jägerskiöld, 1896 ; *Galactosomum phalacrocoracis* Yamaguti, 1939.

As already indicated, the original description of this species was based on metacercariae from cysts in the brain of *Cottus scorpius*. Later (1908), Jägerskiöld found young adult worms in the intestine of every cormorant examined by him in Sweden. Apart from stating the size of the eggs and noting that his specimens agree morphologically with the encysted form, he does not describe these specimens, nor has a detailed description been available hitherto of adults from cormorants inhabiting European seas.

The following description is based on material determined by Professor Jägerskiöld from a cormorant (*Phalacrocorax carbo*) in Sweden, and on material obtained from a shag (*Phalacrocorax aristotelis*) in England. Both sets of material are in the helminthological collection of the British Museum (Natural History).

The body is more or less cylindrical, occasionally widened and somewhat flattened anteriorly. It varies between 1.3 mm. and 3 mm. in length and between 0.3 mm. and 0.5 mm. in maximum width. Scale-like cuticular spines apparently occur only in the anterior half of the body. They are extremely small and show a closely-set quincuncial arrangement. In some specimens, however, the spines appear to be lost, so that the entire cuticle is smooth. The oral sucker is subterminal and measures 0.18 mm. to 0.22 mm. in diameter. The prepharynx is short, measuring up to 0.09 mm. in length, but occasionally it appears to be absent. The pharynx is well developed and elongate, measuring 0.09–0.12 mm. × 0.045–0.06 mm. An oesophagus was not made out. From the pharynx, the intestinal caeca extend laterally or antero-

laterally for a short distance and then turning sharply posteriorly, extend into the hinder region of the body. In the parenchyme between the intestinal caeca, behind the bifurcation, lie numerous unicellular glands, which appear to open into the pharynx, although this cannot be stated with certainty, owing to the poor histological condition of the material available. The excretory vesicle is tubular, extending anteriorly between the testes to the ovary, so that its anterior half has a sigmoid appearance.

The genital atrium is situated a little in front of the middle of the body, and almost constantly measures 0.16 mm. in diameter. In structure it agrees very well with that described by Jägerskiöld for the encysted form. The spacious atrium contains two structures, a "zungenförmigen Körper" or "gonotyl," which can be protruded through the genital aperture, and a thick muscular pad ("sphäroiden Körper")—a reduced ventral sucker—which is provided with numerous very small bristles. The gonotyl lies on the left side of the atrium and the muscular pad on the right. The common genital canal opens into the left wall of the atrium, close to the base of the gonotyl. According to Jägerskiöld, the gonotyl is coated with very small spines, but these were not observed in the present material. When the gonotyl is protruded through the genital pore its free end is flattened and much widened, but when withdrawn into the atrium the free end is comparatively narrow, and the structure has a bluntly conical appearance.

The testes are rounded or oval and measure 0.13–0.24 mm. in length and 0.15–0.22 mm. in width. They lie obliquely one behind the other, the anterior slightly to the left and the posterior to the right of the median line. Infrequently, however, they are arranged distinctly tandem. The seminal vesicle is provided with well-developed muscular walls, and varies between 0.15 mm. and 0.25 mm. in length. It is distinctly constricted into two parts, of which, in mature specimens, the distal or anterior is noticeably much longer than the proximal. The pars prostatica is short and the prostatic gland-cells are few. The ductus ejaculatorius is narrow and relatively short, and unites with the metraterm to form a short common canal.

The ovary lies to the right of the median line in the middle region of the body in front of the testes. It is more or less rounded and measures 0.12–0.21 mm. in diameter. The receptaculum seminis lies postero-dorsally to or immediately behind the ovary. It may be as large as or even larger than the latter. Laurer's canal is much convoluted, and opens in the median line dorsally to the ovary. The vitelline follicles are elongate, arranged laterally in a variable number

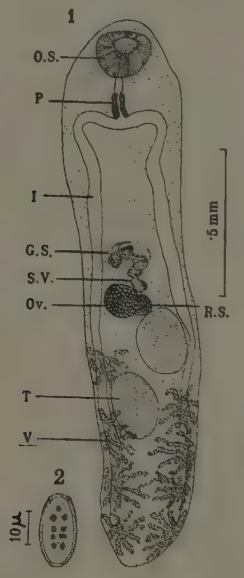
of rosettes, which extend forward about as far as the ovary. Very often the anterior limit is more forward on one side than on the other. Behind the testes the rosettes of follicles, which are extremely difficult to distinguish in macerated material, are confluent dorsally and ventrally in the median line. On leaving the ovarian complex, the uterus runs posteriorly between the testes to fill the hinder region of the body. Its ascending limb also passes between the testes, then between the anterior testis and the ovary, finally crossing the constriction of the seminal vesicle ventrally and extending to the left side of the genital sinus. When the uterus is fully distended with eggs it envelops the testes, the ovary and the proximal portion of the seminal vesicle, and in this condition its exact course is difficult to trace. The eggs are fairly thick-shelled and measure 0.022–0.026 × 0.011–0.013 mm. Some eggs possess an extremely small knob at the posterior pole, but this feature occurs mainly on newly formed eggs and rarely on the older ones.

Yamaguti (1939) distinguishes his species *G. phalacrocoracis* from the present form solely by the size of the eggs. According to Jägerskiöld, the eggs of *G. lacteum* measure 0.022 mm. × 0.011 mm., whereas in *G. phalacrocoracis* they are 0.024–0.027 mm. × 0.012–0.013 mm. The size of the eggs in the present material shows that the distinction observed by Yamaguti is not valid. The main difference between the description of *G. phalacrocoracis* and that given herein for *G. lacteum* is in the size of the body, which in the former is 4–7 mm. in length and 0.55–0.8 mm. in width. Wlassenko (1931) found in the brain of some Black Sea fishes encysted specimens of *G. lacteum* measuring over 2 mm. in length, and it would not be unreasonable to suppose that these specimens would have reached a length of more than 4 mm. in the final host. The relative proportions of the various organs in *G. phalacrocoracis* are rather similar to those found in the writer's material, and therefore it seems that the size of the body alone can hardly be regarded as specifically important. Thus the two species are here considered to be synonymous.

Galactosomum lacteum, which appears to be a somewhat variable species, may now be defined as follows:

Body 1.3–7 mm. in length and 0.3–0.8 mm. in maximum width, occasionally somewhat spatulate anteriorly. Oral sucker 0.18–0.36 mm. in diameter; prepharynx up to 0.44 mm. in length; pharynx elongate, 0.09–0.16 × 0.045–0.12 mm.; oesophagus absent or very short, up to 0.05 mm. in length. Excretory vesicle passing between the testes and extending to the ovary. Genital sinus 0.16–0.4 mm. in diameter. Seminal vesicle constricted into two portions, the anterior being

distinctly longer than the posterior. Pars prostatica narrow and thin-walled. Testes 0.19–0.5 mm. in length and 0.16–0.35 mm. in width, disposed one behind the other, usually obliquely. Ovary rounded or oval, 0.12–0.24 × 0.12–0.25 mm., situated to right of median line. Receptaculum seminis large, lying postero-dorsally to or immediately behind the ovary. Vitelline glands arranged in rosettes of tubular



Galactosomum lacteum.

Fig. 1. Ventral view of adult (uterus omitted). Fig. 2. Egg.

Abbreviations used in Figs. 1, 3 *and* 4.

O.S., oral sucker; P., pharynx; I., intestine; G.S., genital sinus; S.V., seminal vesicle; Ov., ovary; R.S., seminal receptacle; T., testis; V., vitellaria; Ut., uterus.

follicles, extending anteriorly as far as the foremost testis or the ovary. Often they extend more anteriorly on one side than on the other. Eggs 0.022–0.027 × 0.011–0.014 mm.

Occurrence: Intestine of *Phalacrocorax carbo*, Sweden (Jägerskiöld, 1908); *Phalacrocorax p. pelagicus*, Japan (Yamaguti, 1939); *Phalacrocorax aristotelis* and *Ardea cinerea*, Gt. Britain (Baylis, 1939).*

* The writer has been able to confirm the identification arrived at by Dr. H. A. Baylis of immature specimens from the heron.

Metacercariae encysted in brain of *Cottus scorpius*, Sweden (Jägerskiöld, 1896) ; *Cottus bubalis*, Scotland (Nicoll, 1915) ; *Onos tricirratus*, *Smaris chryselis* and *Blennius* sp., Black Sea (Wlassenko, 1931).

2. *Galactosomum semifuscum* (Olsson, 1876).

Synonym : *Monostoma semifuscum* Olsson, 1876.

Although the only description of this form known to the writer, that of Olsson, is very incomplete, there seems to be no doubt that *Monostoma semifuscum* is congeneric with *Galactosomum lacteum*, and Jägerskiöld (1908) has suggested the possibility of the two species being identical. Apparently the main difference between them is in the size of the eggs, which in *G. semifuscum* is said to be 0.018 mm. × 0.011 mm. This difference seems to be rather important, because the present writer has measured innumerable eggs of *G. lacteum* and has never found them to be less than 0.022 mm. × 0.011 mm. The obvious inference is that the eggs in *G. semifuscum* are more rounded. On the other hand, it is possible that the length given by Olsson was obtained from eggs which were tilted in optical view, so that the length appeared less than it should have been. Jägerskiöld found the type-specimens of *G. semifuscum* in a dried-up condition, and it would appear that only by an examination of new material from the type-host, *Sula bassana*, could the question as to the identity of this form with *G. lacteum* be settled satisfactorily.

On the basis of Olsson's description and approximate measurements taken from his figures it is possible to give the following as the more important features of *G. semifuscum* :

Body 3–5.5 mm. in length, and 0.6–1 mm. in width. Oral sucker 0.3 mm. in diameter ; prepharynx and pharynx 0.3 mm. and 0.16 mm. in length respectively. Testes rounded, 0.22 mm. in diameter, tandem. Ovary 0.16 mm. in diameter, situated to right of median line. Vitelline follicles, shape and arrangement not known, apparently extending anteriorly to ovary. Eggs 0.018×0.011 mm.

Occurrence : Intestine of *Sula bassana*, Sweden (Olsson, 1876).

3. *Galactosomum erinaceus* (Poirier, 1886) *emend.*

Synonymy : *Distomum erinaceum* Poirier, 1886 ; *Astiotrema erinacea* Stossich, 1904.

Poirier's description was based on immature specimens obtained from cysts found lying free in the intestine of a dolphin. Jägerskiöld (1908) remarked that these specimens appeared to be very similar to his original material of *Galactosomum lacteum* obtained from cysts in the brain of *Cottus scorpius*. Because of this similarity, he suggested that the cysts of *Distomum erinaceum* had been ingested with fish eaten

by the dolphin, and that the normal final host of this parasite was, in all probability, a fish-eating bird. This form certainly does appear to be conspecific with *G. lacteum*, but a very critical comparison of Poirier's type-specimens with encysted specimens of *G. lacteum* is necessary before the problem can be resolved satisfactorily, especially as the nomenclature of the type-species is also concerned.

According to the original description, based on immature specimens, *Galactosomum erinaceus* may be briefly described as follows :

Body 3 mm. in length and 0.8 mm. in width, anterior region broader than posterior. Cuticle beset with spines. Oral sucker 0.3 mm. in diameter ; prepharynx distinct ; pharynx more or less globular, 0.017 mm. in diameter. Excretory vesicle passing between the testes and the ovary. Seminal vesicle slender, with thick muscular walls. Testes 0.3 mm. in diameter, arranged obliquely one behind the other. Ovary 0.15 mm. in diameter, situated to the right of the median line. Receptaculum seminis large, lying immediately behind the ovary. Vitelline glands not yet developed.

Occurrence : in cysts lying free in intestine of *Delphinus delphis*, locality not known (Poirier, 1886).

4. *Galactosomum cochleariforme* (Rudolphi, 1819).

Synonymy : *Distoma cochleariforme* Rudolphi, 1819 ; *Distomum cochleariforme* Diesing, 1850 ; *Microlistrum cochleariforme* Braun, 1901.

Under the name of *Distoma cochleariforme*, Rudolphi described this form from the intestine of a frigate-bird, *Pelecanus aquila*, and he regarded as belonging to the same species a smaller form from *Sterna* spp. Diesing (1850), however, considered the smaller form to be distinct from that in *Pelecanus aquila* and proposed for it the name *Distomum cochlear*. Braun (1901, 1902), re-examining Rudolphi's material, agreed with Diesing's conclusion regarding the form from terns, and included the two species in a new genus, *Microlistrum*, with *Distoma cochleariforme* as its type. Pratt (1911) obtained further material from *Fregata* [*Pelecanus*] *aquila* and gave a detailed redescription of the species, which he referred to *Galactosomum*.

From the accounts given by Braun and Pratt, *Galactosomum cochleariforme* may be defined as follows :

Body 7–9 mm. in length. The forebody is somewhat widened, measuring 2–2.5 mm. in length and 1.5 mm. in width, whilst the hindbody is more or less cylindrical, measuring 5–6.5 mm. and about 1 mm. respectively. Oral sucker 0.2–0.26 mm. in diameter ; prepharynx short ; pharynx elongate, 0.23×0.16 mm. Excretory vesicle extending

to posterior testis. Genital sinus 0.13–0.16 mm. in diameter. Testes, 0.5–0.67 × 0.35–0.57 mm., arranged tandem. Seminal vesicle constricted at its middle into a very muscular anterior and a thin-walled posterior portion. Ovary about 0.3 mm. in diameter, situated in median line. Receptaculum seminis immediately behind ovary. Vitelline glands arranged in rosettes of very elongate follicles, extending anteriorly as far as the ovary. Eggs 0.022–0.027 × 0.014 mm.

Occurrence : Intestine of *Fregata aquila*, Brazil (Rudolphi, 1819), and Florida, U.S.A. (Pratt, 1911, and Manter, 1930) ; *Fregata magnificens*, Florida (Linton, 1928) ; *Larus argentatus*, Macedonia (Joyeux and Baer, 1928 [not described]).

Witenberg (1929, p. 223) identifies Linton's single specimen from *Fregata magnificens* with *G. spinetum* Braun, but as the specimen is immature this point is disputable, and Linton's identification is here accepted.

5. *Galactosomum cochlear* (Diesing, 1850).

Synonymy : *Distoma cochleariforme* Rudolphi, 1819, p.p. ; *Distomum cochlear* Diesing, 1850 ; *Distomum cochlear sternae* Diesing, 1850 ; *Distomum diesingii* Cobbold, 1861 ; *Microlistrum cochlear* Braun, 1901.

The history of this species has already been given in the discussion on *G. cochleariforme*, and it now remains to mention the specific characteristics.

Body 3.3–5.7 mm. in length. According to Braun (1902), the shape of the body resembles that of *G. cochleariforme*, the forebody being relatively longer and narrower, measuring 0.6 mm. in width, while the hindbody is 0.3–0.46 mm. in width. In one of the figures given by Braun, however, the body is constricted at about its middle and the widest region of the hindbody is a little greater than that of the forebody. Oral sucker 0.11–0.16 mm. in diameter ; prepharynx about as long as the elongate pharynx, which measures 0.15 × 0.11 mm. Genital sinus about 0.13 mm. in diameter. Seminal vesicle long and coiled. Measurements of testes and ovary not known. Testes tandem. Ovary situated in median line. Receptaculum seminis immediately behind ovary. The vitelline glands, according to Braun's figures, are disposed in small rounded follicles, extending anteriorly as far as the receptaculum seminis. Eggs 0.027–0.032 × 0.014–0.016 mm.

Occurrence : Intestine of *Sterna albifrons* [*minuta*], *Sterna* ? *sandvicensis* [*cantiaca*] and *Sterna* sp., Brazil (Rudolphi, 1819 and Braun, 1902) ; *Larus argentatus michahellis*, France (Timon-David, 1934).

Timon-David's record of this species from a herring-gull should be accepted with some reserve. This author gave a meagre description and a very poor figure of his material. He figured a very definite and conspicuous ventral sucker, gave no indication of the distribution of the vitelline follicles, and stated that the eggs measured 24μ × 12μ.

Braun (1902, p. 59) provisionally assigned to *Microlistrum cochlear* two specimens from *Sterna galericulata* [= *maxima*] in the Vienna Museum. Apparently these specimens measure about 3 mm. in length. The anterior region of the body is " tongue-like," measuring 0.6 mm. in length and 0.22 mm. in width, while the remainder of the body is cylindrical, with a maximum width of 0.25 mm. Oral sucker 0.052 × 0.073 mm. ; prepharynx as long as the elongate pharynx, which measures 0.073 × 0.057 mm. Genital sinus 0.069 mm. in diameter. Seminal vesicle 0.3 mm. in length. Vitelline follicles extending anteriorly as far as the receptaculum seminis. Eggs 0.018 × 0.009 mm.

It will be noticed that the eggs are much smaller than those of the typical form and Braun's expressed doubt as to whether these specimens are really *G. cochlear* seems to be justifiable.

6. *Galactosomum spinetum* (Braun, 1901).

Synonym : *Microlistrum spinetum* Braun, 1901.

As will be seen from the characters briefly enumerated below, this species bears a considerable resemblance to *G. cochleariforme*, from which it appears to differ mainly in the greater anterior extent of the vitelline follicles and the uterine coils, and in the position of the constriction of the seminal vesicle. Since so little is known of morphological variation occurring in *G. spinetum*, it cannot be stated with any degree of certainty that these differences are constant.

Body 5–5.5 mm. in length and 1–1.3 mm. in maximum width, which occurs in the anterior region. Oral sucker 0.27–0.3 mm. in diameter ; prepharynx short ; pharynx elongate (0.23 mm. × 0.14 mm.) or rounded (0.2 mm. in diameter). Genital sinus 0.13 mm. in diameter. Measurements of testes and ovary not available. Testes arranged more or less tandem. Seminal vesicle bipartite. Vitelline follicles arranged in rosette-like groups, extending anteriorly beyond the ovary. In contrast to all other species of this genus, the uterine coils extend anteriorly beyond the genital sinus. Eggs 0.022–0.023 × 0.011 mm.

Occurrence : Intestine of *Rhynchops nigra*, Brazil (Braun, 1901 ; 1902).

7. *Galactosomum fregatae* sp. nov.
(Figs. 3 to 5)

Some years ago two specimens of a frigate-bird (*Fregata magnificens rothschildi*) were received at the British Museum (Natural History) from Trinidad, West Indies, having been sent to England in cold storage. While dissecting these birds the writer found in the intestines of both a number of trematodes, which, although not in very good condition, appear to represent a new species of *Galactosomum*.

The length of the mature worms varies between 1.3 mm. and 1.5 mm. and the maximum width between 0.22 mm. and 0.25 mm. The forebody is tapering anteriorly and concave ventrally, while the hindbody is more or less cylindrical. With one exception, all the specimens examined are without spines, probably owing to maceration of the cuticle. When present, the spines are very small and appear to be confined to the anterior region of the body.

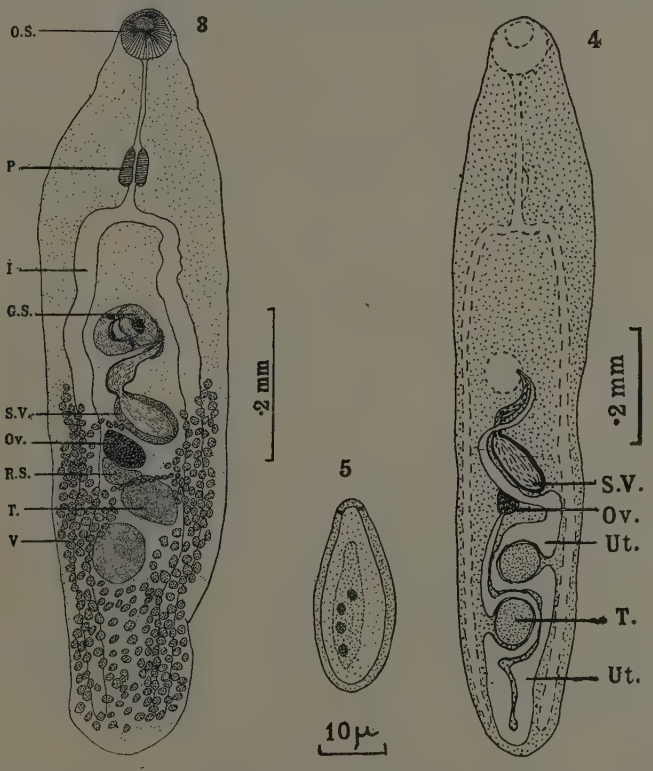
The oral sucker is subterminal and measures 0.075–0.09 mm. in diameter. It opens into a relatively long prepharynx, varying between 0.11 mm. and 0.25 mm. in length. The pharynx is oval, measuring 0.062–0.082 mm. in length and 0.037–0.05 mm. in width. Often an oesophagus cannot be detected, and when this is possible it is very short. The intestinal caeca extend into the hinder region of the body. The excretory vesicle extends anteriorly only as far as the hinder margin of the posterior testis.

The characters of the genital sinus are typical of the genus. The sinus occurs in the middle region of the body and measures 0.075–0.11 mm. in diameter. It contains, attached to its right wall, a large muscular pad ("spheroidal body"), provided with extremely small spines or bristles which appear to be disposed in three groups. A large muscular papilla (" tongue-like body " or " gonotyl ") is attached to the left wall of the sinus, and possesses a covering of spines or bristles at its free end.

The testes are situated either directly or obliquely one behind the other in the middle region of the posterior half of the body. When they are obliquely disposed, which is the less frequent arrangement, the anterior testis lies somewhat to the left, and the posterior to the right of the median line. They are more or less rounded and measure 0.075–0.1 mm. in diameter. The seminal vesicle is a well-developed, somewhat arcuate structure, measuring about 0.15 mm. in length. It is constricted at its middle into a large, highly muscular posterior and a narrow, thin-walled anterior portion. There is a narrow pars prostatica surrounded by a few prostatic gland-cells. A very short ejacu-

latory duct unites with the metraterm to form a common canal which opens through the left wall of the genital sinus, close to the genital pore.

The ovary is situated to the right of the median line, anteriorly to the foremost testis. It is oval or rounded and measures 0.037–0.075 mm. × 0.034–0.067 mm. Between the ovary and the anterior testis



Galactosomum fregatae n. sp.

Fig. 3. Ventral view of adult (uterus omitted). Fig. 4. Adult, showing the course of the uterus in ventral view (semi-diagrammatic). Fig. 5. Egg.

lies a well-developed receptaculum seminis, which may be quite as large as the former. The vitelline glands are disposed in numerous rounded or oval follicles, extending anteriorly to about midway between the ovary and the genital sinus. Anteriorly they are mainly confined to the lateral regions of the body, but behind the testes they are confluent

in the median line. The general course of the uterus appears to be constant, and is precisely similar to that described above for *Galactosomum lacteum*. The eggs are brown-shelled, and the opercular pole is comparatively narrow (Fig. 5). They measure 0.03–0.032 × 0.013–0.015 mm. Well over one hundred eggs, in various regions of the uterus of several specimens, have been measured either in lactophenol or in Canada balsam, and it is interesting to note that the variation between the maximum and minimum measurements, both in length and in width, was only 2μ.

This species rather closely resembles *G. cochlear* (Diesing), but differs principally in the greater anterior extent of the vitelline follicles, and to a lesser degree in the shape and smaller size of the body, the greater relative length of the prepharynx and the shape of the seminal vesicle.

Briefly, *G. fregatae* may be characterized as follows :

Body 1.3–1.5 mm. in length and 0.22–0.25 mm. in maximum width. Forebody tapering anteriorly and concave ventrally ; hindbody cylindrical. Oral sucker 0.075–0.09 mm. in diameter ; prepharynx 0.11–0.25 mm. in length ; pharynx elongate oval, measuring 0.062–0–082 × 0.037–0.05 mm. Excretory vesicle extending to hinder margin of posterior testis. Genital sinus 0.075–0.11 mm. in diameter. Seminal vesicle constricted into a large muscular posterior, and a narrow thin-walled anterior portion. Pars prostatica narrow and thin-walled. Testes 0.075–0.1 mm. in diameter, disposed tandem or obliquely. Ovary 0.12–0.15 mm. in diameter. Receptaculum seminis large, lying immediately behind the ovary. Vitelline follicles irregularly rounded, extending anteriorly as far as the seminal vesicle. Eggs 0.03–0.032 × 0.013–0.015 mm., opercular pole narrowed.

Occurrence : Intestine of *Fregata magnificens rothschildi*, Trinidad, West Indies (present paper).

Type-specimens in the British Museum (Natural History).

8. *Galactosomum puffini* Yamaguti, 1939.

This species is fairly closely related to *G. lacteum*, but differs from it in the relatively smaller oral sucker, in the possession of a large muscular pars prostatica, and in that the hinder portion of the seminal vesicle is much larger than the anterior portion.

The following are the main characters of *G. puffini :*

Body 1.8–2.4 mm. in length and 0.35–0.46 mm. in width, tapering more gradually anteriorly than posteriorly. Oral sucker 0.045–0.1 mm.

in diameter ; prepharynx 0.12–0.24 mm. in length ; pharynx elongate, 0.045–0.054 × 0.027–0.04 mm. Excretory vesicle passing between the testes and extending anteriorly to the receptaculum seminis. Genital sinus up to 0.13 mm. in diameter. Seminal vesicle muscular, constricted to form a very large posterior and a much smaller anterior portion. Pars prostatica large and muscular. Testes and ovary 0.13–0.19 mm. and 0.1–0.16 mm. in diameter respectively, the former arranged obliquely, the latter situated to right of median line. Receptaculum seminis well developed, transversely elongate, immediately behind ovary. Vitelline glands disposed in rosette-like groups of follicles, extending to ovary or seminal vesicle, sometimes more anteriorly on right than on left side. Eggs 0.021–0.024 × 0.012–0.014 mm., narrower at opercular pole.

Occurrence : Intestine of *Puffinus leucomelas*, Japan (Yamaguti, 1939).

9. *Galactosomum johnsoni* Price, 1934.

This and the next species, *G. darbyi*, differ from all others of the genus in the fact that neither possesses a constriction of the seminal vesicle.

Briefly, *G. johnsoni* may be characterized as follows :

Body 0.97–1.03 mm. in length and 0.28–0.34 mm. in maximum width. Oral sucker 0.06–0.09 mm. in diameter ; prepharynx relatively long, 0.12–0.28 mm. ; pharynx 0.06 × 0.04–0.05 mm. ; oesophagus 0.04–0.06 mm. in length. Seminal vesicle relatively large, without a constriction, but according to Price's original figure the vesicle has a well-marked bulbous hinder and a narrow anterior portion. Testes 0.09–0.12 mm. in diameter, arranged more or less diagonally one behind the other. Ovary transversely oval, 0.04–0.048 × 0.06–0.062 mm., situated to right of median line. Contrary to its position in all other species of the genus, the receptaculum seminis here lies anteriorly to the ovary. Vitelline glands consisting of rounded or oval follicles, which are mainly disposed between and ventrally to the caeca, behind the ovary. Eggs 0.034–0.036 × 0.020 mm., flattened on one side.

Occurrence : Intestine of *Sula leucogastra*, Puerto Rico, West Indies (Price, 1934).

10. *Galactosomum darbyi* Price, 1934.

In the main, this species bears a very close resemblance to the last, from which it may apparently be distinguished by the shape of the seminal vesicle, by the position of the receptaculum seminis in relation to the ovary, and by the shape and size of the eggs.

The following are the more important features of *G. darbyi:*

Body 0.8–0.97 mm. in length and 0.14–0.20 mm. in width, usually with slight constriction near middle region. Oral sucker 0.056–0.06 mm. in diameter; prepharynx 0.16–0.18 mm. in length; pharynx more or less elongate, 0.04–0.044 × 0.02–0.04 mm., oesophagus 0.024–0.04 mm. in length. Seminal vesicle elongate, sigmoid, without a constriction. Testes 0.06–0.1 mm. in diameter, arranged more or less obliquely side by side. Ovary globular or transversely oval, 0.02–0.048 × 0.04–0.06 mm. Receptaculum seminis posterior to ovary. Vitelline glands consisting of rounded or oval follicles, mainly intercaecal and between ovary and hinder end of body. Eggs 0.022–0.024 × 0.012–0.014 mm.

Occurrence: Intestine of *Pelecanus occidentalis occidentalis,* Dominican Republic (Price, 1934).

11. *Galactosomum humbargari* Park, 1936.

This species is apparently very closely related to *G. darbyi,* from which it differs mainly in the larger size of the body and in the presence of a constriction in the seminal vesicle, both of which may be arbitrary features from a systematic point of view. However, the following are the more important features of *G. humbargari:*

Body 1.8–3 mm. in length and 0.27–0.46 mm. in width, slightly constricted near middle region. Oral sucker 0.064–0.13 mm. in diameter; prepharynx 0.14–0.24 mm. in length; pharynx globular, 0.053–0.075 mm. in diameter; oesophagus up to 0.028 mm. in length. Excretory vesicle extending to posterior testis. Genital sinus 0.09–0.16 mm. in diameter. Seminal vesicle large, with one or two constrictions. Testes 0.068–0.16 mm. in diameter, arranged obliquely one behind the other. Ovary globular, 0.08–0.16 mm. in diameter. Receptaculum seminis immediately behind ovary. Vitelline glands consisting of rounded follicles lying between the intestinal caeca and extending from the ovary to the posterior end of the body. Eggs 0.022 × 0.014 mm.

Occurrence: Intestine of *Larus californicus,* California, U.S.A. (Park, 1936).

12. *Galactosomum aharonii* (Witenberg, 1929).

Synonym: *Cercarioides aharonii* Witenberg, 1929.

This species may be readily distinguished from all the foregoing species by the shape of the body and, with the exception of *G. johnsoni,* by the relatively large size of the eggs.

Body 3.4 mm. in length, constricted at about the anterior third into a flattened, broadly-pyriform forebody and a spindle-shaped hindbody, which contains the genital organs. Oral sucker 0.38 mm. in diameter; prepharynx very short, surrounded by glandular cells; pharynx globular, 0.14 mm. in diameter. Genital sinus at junction of fore and hind portions of body. Seminal vesicle constricted. Testes slightly lobed, about 0.38 mm. in diameter, arranged obliquely one behind the other. Ovary 0.18 mm. in diameter. Vitelline glands consisting of numerous large, irregularly-shaped follicles, scattered throughout the posterior region of the body behind the ovary. Eggs 0.037 × 0.022 mm.

Occurrence: Intestine of *Puffinus kuhli*, Egypt (Witenberg, 1929).

18. *Galactosomum baylisi* (Nazmi, 1930).

Synonym: *Cercarioides baylisi Nazmi*, 1930.

This species bears a considerable resemblance to *G. aharonii* (Witenberg). Regarding the morphology of these species, only the information contained in the original descriptions is at present available. Each description is based on a single specimen, and it is therefore quite possible that when further material is examined the species will prove to be identical. Briefly, *G. baylisi* may be characterized as follows:

Body 7.5 mm. in length, bearing a considerable resemblance to *G. aharonii* in shape. The pear-shaped anterior portion measures 3 × 1.7 mm., while the spindle-shaped hindbody measures 4.5 × 1.25 mm. Oral sucker 0.55 × 0.73 mm.; prepharynx short; pharynx apparently about half as large as oral sucker. Genital pore situated anteriorly to constriction of body. Genital sinus 0.27–0.33 mm. in diameter. Seminal vesicle, 0.45 mm. in length and 0.15 mm. in width, constricted at about its middle. Testes slightly lobed, 0.75 mm. in diameter, disposed obliquely one behind the other. Ovary rounded, 0.25 mm. in diameter. Vitelline glands consisting of irregularly-shaped follicles, widely distributed between intestinal caeca in posterior half of body. They extend forward as far as the anterior limit of the foremost testis on the right and that of the hinder testis on the left side. Eggs 0.045 × 0.02 mm., narrowing somewhat towards opercular pole.

Occurrence: Intestine of domestic goose (*Anser anser dom.*), Egypt (Nazmi, 1930).

KEY TO SOME SPECIES OF THE GENUS *Galactosomum*.

I. Vitelline follicles lateral to intestinal caeca (confluent in median line behind testes).

A. Vitelline follicles elongate, arranged in a number of rosette-like clusters.

1. Seminal vesicle constricted to form a large, muscular, posterior portion and a smaller, less muscular, anterior portion.

a. Genital sinus about half as large as oral sucker; pars prostatica narrow, poorly developed; uterine coils extend anteriorly beyond genital sinus.....*spinetum*

b. Genital sinus as large as or larger than oral sucker; pars prostatica bulbous, well developed; uterine coils not extending beyond genital sinus.....*puffini*

2. Seminal vesicle constricted to form a small posterior portion and a very large anterior portion.

a. Pharynx very much smaller than oral sucker.....*lacteum*

b. Pharynx as large as oral sucker.....*cochleariforme*

B. Vitelline follicles rounded or oval, not arranged in clusters or special groups.

1. Seminal vesicle with several spiral turns; vitelline follicles extending anteriorly to receptaculum seminis, but not anteriorly to seminal vesicle.....*cochlear*

2. Seminal vesicle without spiral turns; vitelline follicles extending anteriorly to seminal vesicle.....*fregatae*

II. Vitelline follicles between intestinal caeca.

A. Body flattened and broadly pyriform anteriorly, relatively very narrow and cylindrical posteriorly.

1. Seminal vesicle constricted at about its middle; eggs $45\mu \times 20\mu$.....*baylisi*

2. Seminal vesicle constricted to form a large posterior portion and a very small rounded anterior portion; eggs $37\mu \times 22\mu$ *aharonii*

B. Body not divided into two distinct portions.

1. Eggs $34–36\mu \times 12–14\mu$, flattened on one side.....*johnsoni*

2. Eggs $22–24\mu \times 12–14\mu$, symmetrical.

a. Seminal vesicle with one or two constrictions..*humbargari*

b. Seminal vesicle not constricted.....*darbyi*

14. *Galactosomum anguillarum* (Tubangui, 1933).

Synonym : *Haplorchis anguillarum* Tubangui, 1933.*

The original description of *G. anguillarum* was based on a single specimen from the intestine of an eel. Possibly the intermediate host may have been a fish swallowed by the eel, but Tubangui and Africa (1938) doubted whether any species of *Galactosomum* would develop to full maturity in fishes. Until evidence to the contrary is forthcoming, the occurrence of a species of this genus in the intestine of an eel should be regarded as accidental. Briefly, *G. anguillarum* may be described as follows :

Body 2.9 mm. in length and 0.56 mm. in maximum width. Oral sucker 0.15 × 0.18 mm. ; prepharynx 0.34 mm. in length ; pharynx 0.14 × 0.16 mm. Genital sinus 0.1 × 0.13 mm. Seminal vesicle constricted into a large posterior and a small anterior portion. Pars prostatica narrow. Testes spherical, 0.24–0.26 mm. in diameter, arranged one directly behind the other. Ovary 0.14 × 0.17 mm. Receptaculum seminis immediately behind ovary. Vitelline follicles somewhat dendritic, situated laterally, mainly behind posterior testis, but not reaching to ends of intestinal caeca. Eggs not yet developed.

Occurrence : Intestine of *Anguilla mauritiana*, Philippines (Tubangui, 1933).

With the exception of the little-known or immature forms *G. semifuscum*, *G. erinaceus* and *G. anguillarum*, it appears possible to divide the species of *Galactosomum* into two main groups, according to the distribution of the vitelline follicles. The species within each group may be distinguished principally by the shape and arrangement of the follicles and by the position of the constriction in the seminal vesicle. In connection with these features there are one or two points that have been observed in the writer's material of *G. lacteum* and *G. fregatae* which must be mentioned. In macerated specimens of *G. lacteum* the vitelline follicles, normally elongate and disposed in rosette-like clusters in well-preserved material, are broken up into small, irregularly-shaped masses, having no definite arrangement. Further, in both species the position of the constriction in the seminal vesicle is constant when the testes are fully active, but as the production of spermatozoa declines the seminal vesicle contracts. In the latter condition the constriction is sometimes extremely difficult to make out, and the relative size of the two portions is considerably altered,

* Srivastava (1935) assigned this species to *Tubanguia*, but did not make the combination of generic and specific names.

often so much that in some of the writer's specimens of *G. lacteum* the hinder portion has apparently disappeared. It should be noted, however, that the constriction is sometimes obscured by the uterine coils which cross the seminal vesicle ventrally. In such cases the exact position of the constriction, if present, may be determined by an examination of the vesicle in dorsal view. In view of these observations, it is apparent that when identifying specimens belonging to this genus their condition should be taken into consideration.

The importance placed on the above-mentioned features as a means of distinguishing the species of *Galactosomum* might be considered questionable. Thus the foregoing differential key, from which *G. semifuscum*, *G. erinaceus* and *G. anguillarum* are omitted, should be regarded as a tentative one, for it is intended primarily to show that certain features may prove to be of real systematic value when some of the species are better known. On the other hand, the key also shows that some species are very closely related and difficult to separate satisfactorily, and it is possible that they may subsequently be shown to be identical.

To conclude the present work, it may be useful to tabulate the species of *Galactosomum*, together with the hosts from which they have been recorded. *G. erinaceus* (Poirier, 1887) and *G. anguillarum* (Tubangui, 1933) are omitted from this table, because the hosts in which they were found were probably accidental.

Parasite	*Host*
G. aharonii (Witenberg, 1929)	Procellariiformes
	Puffinus kuhli
G. baylisi (Nazmi, 1931)	Anseriformes
	Anser anser dom.
G. cochlear (Diesing, 1850)	Lariformes
	Sterna albifrons
	Sterna sandvicensis
	Sterna maxima
	Larus argentatus michahellis
G. cochleariforme (Rud., 1819)	Pelecaniformes
	Fregata aquila
	Fregata magnificens
	Lariformes
	Larus argentatus
G. darbyi Price, 1934	Pelecaniformes
	Pelecanus o. occidentalis

G. fregatae n.sp.	Pelecaniformes *Fregata magnificens rothschildi*
G. humbargari Park, 1936	Lariformes *Larus californicus*
G. johnsoni Price, 1934	Pelecaniformes *Sula leucogastra*
G. lacteum (Jägersk., 1896)	Pelecaniformes *Phalacrocorax carbo* *Phalacrocorax aristotelis* *Phalacrocorax p. pelagicus* Ardeiformes *Ardea cinerea*
G. puffini Yamaguti, 1939	Procellariiformes *Puffinus leucomelas*
G. spinetum Braun, 1901	Lariformes *Rhynchops nigra*

Summary.

1. The genera *Galactosomum*, *Microlistrum* and *Stictodora* are compared, and, while the first two are undoubtedly synonymous, the last is considered to be distinct.

2. The adult of the type-species of *Galactosomum*, *G. lacteum*, is fully described for the first time from European cormorants, and *G. phalacrocoracis* is regarded as a synonym.

3. A new species, *Galactosomum fregatae*, is described from the frigate-bird *Fregata magnificens rothschildi*.

4. The characters of all the species that have been assigned to *Galactosomum* are briefly defined, and a provisional differential key is given.

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Investigations on the Emergence of Larvae from Cysts of the Potato-root Eelworm *Heterodera rostochiensis*.

1. Technique and Variability.

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Whilst it is a well-known fact that the emergence of the larvae of *Heterodera rostochiensis* from the cyst under natural conditions is conditional upon the presence of an actively growing potato or tomato crop and that substances are given off from the host plant which are responsible for this stimulation, relatively little is known regarding the intimate details of the process. This, in the author's opinion, is due in no small degree to the difficulty of obtaining consistently reproducible results. Only too often in the past has the writer carried out an experiment and obtained an apparently clear-cut result to find on repeating the experiment, a month or so later, that the second result is completely different. The importance of gaining all possible information regarding the process cannot be over emphasized when it is considered that larval emergence constitutes the first step in the process culminating in the entry of the parasite into a new host. Furthermore, since there is to date no completely reliable method of estimating the viability of cysts except by stimulation with diffusates of potato or tomato roots then the necessity of standardising the process becomes apparent. The present work, of which this paper records a part, represents an attempt to investigate the different factors influencing larval emergence with a view to making possible the conduct of experiments with a reasonable chance of obtaining consistent results.

One is led at this stage to speculate on the many variables which influence larval emergence when cysts are immersed in solutions of root diffusate under laboratory conditions. Arguing from first principles, it would appear that there are at least three main factors influencing hatching, the cysts themselves, the root diffusate and the physical conditions during larval emergence as well as prior to it. Preliminary experiments did, in fact, show that all three factors had a very considerable effect on hatching, which was manifested both in the varying numbers of larvae obtained, as well as in the time taken for these larvae to emerge. Separate samples of cysts drawn from the same stock exhibited very considerable variation, whilst samples

taken from different stocks frequently behaved completely differently from one another. One fact emerged at a very early stage—it was imperative when once hatching had commenced that it should be allowed to proceed to completion. Further, any completely negative result had to be accepted with caution and the cysts removed and exposed again a few months later, since it was found to be not at all uncommon for the effect of different chemicals to be manifested by a prolonged delay in hatching. Humidity and temperature also had an effect on the subsequent hatching of " air dried " cysts when exposed to root diffusate. Different samples of root diffusate varied greatly in their hatching properties whilst temperature and other physical conditions were not without their effect.

The experiments described herein are concerned primarily with investigating the inherent variability of the experimental material, namely cysts of *Heterodera rostochiensis*, and to this end, as will be seen from the description of technique, all other factors have been kept as constant as possible.

Technique and Material.

In order to obtain a diffusate which was relatively standard throughout an experiment, as well as from one experiment to another, efforts were directed mainly towards building up a large store of this substance which could be drawn on as and when desired. The necessity for this was apparent in earlier experiments where it was found that when root diffusate was taken and used fresh, hatching was most irregular and completely unpredictable. This irregularity was attributed to several different factors which were only of importance is so far as they influenced the activity of the different samples of root diffusate collected. In point of fact it was found that the quality of a root diffusate sample could be influenced by day to day temperature fluctuations, degree of leaching carried out, as well as the frequency of watering. While it is not intended to pursue the effect of these different factors in the present paper, the writer would merely call attention to them in order to justify his attempts at securing a relatively constant extract.

The plants used for the extract collected in these experiments were potatoes of the variety Arran Banner. A 1:4 mixture of sand and good quality unsterilized medium loam sieved through a $\frac{1}{4}$-in. mesh sieve was put up in 6-in. pots. No peat was added to the mixture, nor were artificials or lime used. Uniform sized chitted tubers were planted in this mixture and grown in a cool house. During the early stages of

growth the plants were watered normally and the roots examined periodically by inverting a pot and knocking out the soil mass. Leaching for root diffusate was commenced when a moderately dense mass of roots was found encircling the soil mass, i.e. when the plants were nearly pot bound. Watering was reduced to a minimum when leaching was commenced. The plants generally were found to grow quite well under these conditions, the mixture of loam and sand being apparently quite suitable for the growth of potatoes.

The arrangements were those in general use at the late Institute of Agricultural Parasitology, St. Albans. Tables were covered with sheets of asbestos supported on wooden bearers in such a way that they drained into sloping asbestos gutters. The pots were stood on the asbestos sheets and the leachings were thus discharged directly from the gutters into enamel pails.

The quantity of water used for leaching was carefully regulated. Six plants were taken at random and 50 cc. of water were added to each. This was followed by another 50 cc., the process being repeated until the pots were saturated. The average quantity necessary to saturate the six pots was estimated and double this quantity was added to each of the remaining plants. Leaching was never carried out oftener than twice per week.

Later experiments have indicated that the amount of water used (double the saturation deficit) was excessive. A better diffusate might have been obtained if not more than 150% or even 120% of the saturation deficit had been added. In any case it is highly doubtful whether the saturation deficit is the correct measure for determining the quantity of water to be used. It would appear probable that a better criterion would be a constant volume in excess of the saturation deficit and in later experiments this method was resorted to. Results have confirmed this view in that smaller quantities of root diffusate of apparently greater potency have been obtained. A further refinement has been the addition of about 90% of the saturation deficit to be followed about 30 minutes later by the other 10% plus the excess. This latter method is now the standard method for the collection of root diffusate in this Department.

Root diffusate collected by this method was filtered through No. 1 Whatman filter paper and stored in a refrigerator at a temperature of 0–4° C. Under these conditions the loss inactivity over a period of up to 3 years was not sufficient to be detected, and it has not proved to be at all uncommon for a three-year old sample of diffusate to be equally if not more active than a fresh sample.

The hatching technique was that described by the author in a previous paper (Fenwick, 1943). The cysts were put up in the single cyst chambers in root diffusate which was changed weekly. The temperature of incubation was 24°C. and root diffusate from the same stock was used throughout each experiment. Prior to hatching, the cysts were soaked for three weeks in tap water and for convenience, only those cysts which had sunk were used for experiment. The effect of this was to bias the selection a little in favour of full cysts at the expense of empty ones, since it was found that cysts which still floated after a period of more than one week's soaking were invariably empty. To this extent the cyst populations studied were not normal. Hatching was continued until no more larvae emerged, at which stage the cysts were dissected with needles, and the number of larvae and apparently full eggs left in each cyst were counted.

Three different batches of cysts were used as material, being by-products of three experiments on chemical control. Referring to Tables 1, 3 and 5, batches 1 and 2 included 4 treatments each, A–D and E–H respectively, while batch 3 represented seven treatments I–O. A, E and H represented control cysts whilst the rest were treated material. In the case of batches 1 and 2 the treatment effects were known to be slight, whilst in batch 3 there was known to be a high mortality amongst some of the treated cysts ; 50 individual cysts were put up to represent each treatment.

Aims, Procedure and Results.

The aim of these experiments, as indicated earlier, was to obtain information regarding the inherent variability of cysts, as well as to determine the best method of analysing the data obtained. No attention was paid to the rate of hatching nor to the form of the hatching curve. It is hoped to deal with the latter point in a later paper. Accordingly, the only data recorded were the total number of larvae emerging from each cyst and the total of eggs and larvae left within the cyst. The two figures added together gave the total number of eggs and larvae originally present.

Consideration of space preclude the possibility of publishing the raw data, but Table 1 gives data relating to the mean and variance of larvae hatching from each cyst for each separate treatment. The term σ_{100} in this table refers to the standard deviation of mean hatches from 100 cysts, and the ratio $\sigma/\bar{x}$ gives a measure of the variability inherent in a sample of cysts expressed as a proportion of

the mean—i.e. the coefficient of variation. Perusal of this table indicates at once the very high degree of variability present, the standard deviation of the number of larvae emerging from one cyst being of the same order as the mean, the general ratio for $\sigma_1/\bar{x}$ being 1.05. A very disquieting feature indicated by the values of $\sigma/\bar{x}$ is the

TABLE I.

Variability Table for Untransformed Data.

	$\bar{x}$	σ^2	σ_1	$\sigma/\bar{x}$	$\sigma_{100}/\bar{x}$
A	316.4	41,066	202	0.64	0.064
B	165.5	18,289	135	0.82	0.082
C	224.6	25,056	159	0.70	0.070
D	222.2	41,351	204	0.92	0.092
E	313.6	89,272	299	0.96	0.096
F	206.9	46,101	215	1.04	0.104
G	202.4	35,798	189	0.94	0.094
H	266.1	29,624	172	0.65	0.065
I	57.8	2,138	46.2	0.80	0.080
J	27.9	1,678	41.0	1.47	0.147
K	27.2	1,746	41.8	1.54	0.154
L	34.3	5,332	73.0	2.12	0.212
M	9.2	202	14.2	1.54	0.154
N	21.1	718	26.9	1.27	0.127
O	15.4	588	24.2	1.57	0.157
General ..	140.5	21,614	147	1.05	0.105

very large sized sample needed to secure anything like a reasonable degree of accuracy. For samples of 25 cysts the coefficient of variation is 21%, whilst even for 100 cysts it is 10.5%, which leads one to the conclusion that it is absolutely imperative, in order to secure a reasonable accuracy, that a sample of, at least, 50 and preferably 100 cysts be used.

Even more worrying than the magnitude of the standard deviation is the comparative constancy of the ratio $\sigma_1 \bar{x}$, which leads one to the conclusion that the variance and mean are very closely associated. Since a significant correlation between these parameters would invalidate an analysis of untransformed data, it is imperative that it be investigated, and Table 2 presents the result of an investigation into this phenomenon. It will be seen that the correlation between variance and mean has been investigated both for treatments within batches, as well as between batches, and also generally. It will be seen that for correlation within batches $t = 3$, $p = 0.02$ and between batches $t = 12$, $p = 0.10$. Since $p = .05$ is the generally accepted level for significance, it might be said that there is a significant correlation between $\bar{x}$ and σ^2 both within batches and generally but the conditions are not sufficiently sensitive to indicate the "between batches" correlation. The correlation between $\bar{x}$ and σ, however, proved to be

TABLE II.

Correlation Studies for Untransformed Data.

	D. of F.	SS(x) (x = mean)	SS(y) (y = variance)	S(xy)	r	S.E. of r	t
Within	9	2[illegible]	2,569,999,723	4,712,427	0.63	.21	3 (Sig. at .02)
Between	1	168,594	6,437,831,341	31,587,497	0.96	.08	12 (Sig. at .10)
Total	10	1[illegible]	9,[illegible]37,831,064	36,299,924	0.876	.066	13.3 (Sig. at .001)

just significant, with $r = 0.98$ with a S.E. of .04 yielding a value of $t = 24.5$. In any case the lack of significance of r for $\bar{x}$ and σ^2 is mainly due to the very high values of t necessitated by there being only one degree of freedom present. In all probability had it been possible to include more batches in the experiment the correlation coefficient between batches would have been significant.

The presence of these significant correlations between σ^2 and $\bar{x}$ which render analysis of untransformed data invalid led to the search for a transformation which would break down or, at least, substantially reduce this unfortunate correlation and two transformations were investigated—a logarithmic and a square root transformation. The results of a logarithmic form are set out in Tables 3 and 4 which correspond to Tables 1 and 2. In this case all individual values were converted to $100 \log (x - 1)$. The first effect of this transformation appears to be a reduction of $\sigma \bar{x}$ from 1.05 for untransformed data to

0.67 for logarithmic data, whilst there is some indication that $\sigma/\bar{x}$ tends to increase as $\bar{x}$ decreases, suggesting that correlation between $\bar{x}$ and σ^2 is being reduced. This indication is confirmed by Table 4, where it will be seen that the several values of t are 2.2, 3.8 and 2.1, whilst $p = 0.1$, $p = 0.2$ and $p = 0.1$—none of which are significant. When

TABLE III.

Variability Table for a Logarithmic Transformation.

	$\bar{x}$	σ^2	σ	$\sigma_1/\bar{x}$	$\sigma_{100}/\bar{x}$
A	219	7,175	84.7	0.39	0.039
B	182	7,639	87.4	0.48	0.048
C	193	9,123	95.5	0.49	0.049
D	149	16,350	128.0	0.86	0.086
E	179	15,580	124.8	0.69	0.069
F	152	14,890	122.0	0.80	0.080
G	157	14,473	120.4	0.77	0.077
H	203	9,479	97.4	0.48	0.048
I	144	15,019	70.8	0.49	0.049
J	99	5,402	73.5	0.74	0.074
K	94	5,529	74.4	0.79	0.079
L	74	7,060	83.0	1.12	0.112
M	64	3,440	58.7	0.92	0.092
N	94	4,763	69.0	0.73	0.073
O	78	3,831	61.9	0.79	0.079
General ..	139	8,640	93.0	0.67	0.067

a similar computation was performed for $\bar{x}$ and σ the general correlation gave a value of $t = 2.16$ with $p = .05$. The conclusion can, therefore, be drawn that a logarithmic transformation of data reduces the coefficient of variation from unity to about 0.66% and very largely eliminates the correlation between $\bar{x}$ and σ^2.

The effect of a square root transformation was a variation coefficient of 0.75—a value intermediate between that for untransformed data and that for the logarithmic transformation. The correlation between $\bar{x}$ and σ^2 were investigated, giving values of $p = 0.7$ for "within batch" values, $p = 0.1$ for "between batch" values and $p = .001$ for all values generally, the values of r being —0.184, +0.96 and +0.815 respectively. The value of r for "between batch" correlation for $\bar{x}$ and σ was 0.975 with $t = 19.5$ and $p = .05$. It would, therefore, appear justifiable to conclude that the square root transformation is less satisfactory than is the logarithmic since it merely reduces and does not eliminate correlations between $\bar{x}$ and σ^2. It is, moreover, a very much more difficult transformation to utilise since means obtained by it are very difficult to interpret and consequently there seems little point in using it.

TABLE IV.

Correlation Studies for a Logarithmic Transformation.

	D. of F.	SS(x) (x=mean)	SS(y) (y=variance)	S(xy)	r	S.E. of r	t
Within	9	8,219	86,496,591	—450,267	—0.535	0.24	2.2 (Sig. at 0.1)
Between	1	28,480	199,249,989	2,102,711	+0.883	0.23	3.8 (Sig. at 0.2)
Total	13	36,699	285,746,580	1,602,444	+0.495	0.06	2.1 (Sig. at 0.1)

Since the very high degree of variability inherent in hatching data renders it necessary to utilise very large numbers of cysts in order to obtain reasonably accurate estimates, it would be interesting to investigate the causes of this variability. Study of the data in its original form indicated that much of the variation in hatching was merely a reflection of the variability in the original contents of the cysts, and the idea of expressing the hatch in terms of the original cyst contents suggested itself. Consequently, the number of larvae hatching was added to the number of eggs and larvae remaining within the cyst and expressed as a proportion of this total. This latter figure was then submitted to the angular transformation and the values of ϕ then obtained were treated as normal ordinates. The results of analysis of the data thus obtained are set out in Tables 5 and 6, which again correspond in form to Tables 1 and 2 respectively. The coefficient of variation will be seen to have been reduced to 0.32% and there is no significant correlation between mean and variance either within batches or generally but the correlation between batches just borders

TABLE V.

Variability Table for Angular Transformation.

	$\bar{x}$	σ^2	σ	$\sigma_1/\bar{x}$	$\sigma_{100}/\bar{x}$
A	82.2	71	8.4	0.10	0.010
B	88.4	8	2.8	0.03	0.003
C	66.0	227	15.1	0.22	0.002
D	70.5	182	13.5	0.19	0.019
E	74.2	189	13.8	0.19	0.019
F	81.8	404	20.2	0.25	0.025
G	85.3	104	10.2	0.12	0.012
H	69.5	60	7.7	0.11	0.011
I	32.9	134	11.6	0.35	0.035
J	43.6	656	25.6	0.59	0.059
K	33.9	378	19.5	0.58	0.058
L	29.4	227	15.2	0.52	0.052
M	16.8	222	14.9	0.88	0.088
N	30.0	479	22.1	0.74	0.074
O	40.1	916	30.3	0.76	0.076
General ..	52.1	248	15.8	0.30	0.030

TABLE VI.

Correlation Studies for Angular Transformation.

	D. of F.	SS(x) (x=mean)	SS(y) (y=variance)	S(xy)	r	S.E. of r	t
Within	9	919	554,778	+7,967	0.354	0.312	1.18 (Sig. at 0.3)
Between	1	7,512	300,682	—45,866	—0.97	0.06	16 (Sig. at .05)
Total	13	8,431	855,460	—37,809	—0.445	0.22	2.0 (Sig. at 0.1)

on the $p = .05$ level of significance. If tests are made between $\bar{x}$ and σ then for the general correlation, $p = .02$, but there is no significant correlation for any other component. It would thus appear that the ϕ transformation is not as satisfactory in attaining independence between mean and variance as was the logarithmic form, although it appears to increase the sensitivity of every result obtained since the coefficient of variation is reduced to approximately 0.32.

To determine how the different transformations affected the sensitivity, analyses of variance were carried out, using all three transformations and summarized results of these are set out in Table 7, a, b, c and d representing untransformed data, logarithmic, square root and angular transformations respectively. In all cases the treatment effects are significant at $p = .001$, as is also the case for batch differences. The actual values of variance ratio, however, show certain apparent peculiarities. The v_2/v_1 ratio, measuring the treatment effect, remained fairly constant at approximately 8 for untransformed data and for the square root transformation, but fell to 3.95 for the logarithmic transformation, while for the ϕ data the value was 9. F for v_3/v_2 was approximately the same for untransformed and logarithmic data, but fell to 16.9 for the square root data and rose to 70 for ϕ data. The ϕ transformation would thus appear to be at least as sensitive as any and there are some indications that it might well be the most sensitive of all.

Discussion.

It would appear from the foregoing that the conduct of hatching experiments is not by any means as simple as one would wish. The very high degree of variability exhibited by the cysts themselves, in the absence of any variables such as root diffusate, etc., renders necessary the greatest care in setting up a hatching experiment, and the question of deciding on the best method of designing such an experiment assumes some importance. It is highly doubtful whether single cyst hatching is an economical use of time, since when plates are put up containing 50 cysts, the mean number of larvae hatching on different plates would be subject to an error of $\pm 15\%$. It would, therefore, appear to be better to hatch out bulk samples of cysts. As this would involve large numbers of larvae, a dilution method might have to be resorted to in counting. Some of the errors applicable to different sized bulk samples can be gathered from Table 1, where it will be seen that bulk samples of 100 cysts are subject to a standard deviation of $\pm 10\%$ and there is no method of statistical analysis which can obviate this central fact. The use of samples larger than 100 cysts,

TABLE VII.

Effect of Different Transformations on the Analysis of Variance.

Source	Variance				Variance ratios				Significance			
	a	b	c	d	a	b	c	d	a	b	c	d
Cysts within treatments (v_1)	21,614	8,640	42.83	248	—	—	—	—	—	—	—	—
Treatments within batches (v_2)	188,907	34,162	348.2	2,238	8.7	3.95	8.14	9.0	.001	.001	.001	.001
Batches (v_3).....	3,959,657	707,152	5,863.5	139,588	21	20.8	16.9	70	.001	.001	.001	.001

desirable though it may be from a statistical point of view, tends to be inconvenient practically since 100 cysts seems to be the maximum number which can be easily accommodated in a solid watchglass. Increased accuracy must, therefore, be attained by replicating the number of batches used per treatment.

The use of a dilution technique for estimating the number of larvae obtained from bulk samples of cysts is a further point requiring attention, since, when larvae are liberated from a batch of cysts and enumerated by dilution there are two sources of variation—that due to the batch used as well as a sampling error in the dilution technique itself. The former is dependent on the size of the sample of cysts used and the present paper furnishes some information regarding the expected magnitude of this error for any sized sample. The latter is dependent only on the number of larvae counted in a sample of diluent and is independent of the size or quality of the cyst sample. Assuming a perfect dilution and sampling technique this latter is also predictable and should correspond to $1/\sqrt{n}$ where n is the number of larvae counted. The importance of designing the sampling technique so that its accuracy is at least as high as that associated with the given size of cyst sample then becomes obvious.

The decision on the best method of handling the data obtained is more difficult. The use of untransformed data is obviously not permissible. Of the three transformations mentioned arguments can be advanced for any one of all three. The logarithmic transformation is obviously the best as far as independence between mean and variance is concerned, but appears to be the least sensitive for picking out treatment effects. The other transformations appear to be not completely satisfactory on the former criterion, but do not appear to have lost sensitivity as compared with untransformed data. In fact, as far as distinguishing between batch effects is concerned the ϕ transformation seems to have gained sensitivity. The square root transformation appears to have no obvious advantage over the logarithmic, on the one hand, or the angular, on the other, and is, moreover, not convenient as far as computation is concerned, since means thus derived are very difficult to interpret, so that the weight of evidence would apparently be in favour of either the logarithmic or the angular. The latter would appear to be the most useful in carrying out experimental work, since it would automatically compensate for any initial differences in the egg content of the experimental material ; moreover, since ϕ is a function of the percentage hatch it would appear to be a more fundamental concept and the apparent increase in sensitivity

resulting from its use might well be one aspect of this fact. The degree of independence between mean and variance thus obtained whilst not complete is probably sufficient for all practical purposes. In any case, if a computation is based on the logarithmic transformation, it involves the basic assumption that more or less equal numbers of larvae are available in each batch for hatching. It is, consequently, of the utmost importance that counts be made when hatching is complete to determine the number of eggs left within the cysts, so that the total number present at the beginning of the hatching will be known, thus verifying the accuracy or otherwise of this assumption.

Summary and Conclusions.

Fifteen lots of fifty individual cysts from three separate batches were exposed to the action of potato root diffusate, the hatching allowed to continue to completion and the total larvae liberated from each cyst were counted. The data obtained were analysed both as they stood and after three different transformations for variability, as well as for independence between mean and variance. Very high variability corresponding to a coefficient of variation of 1.00 was detected, and there was, moreover, a very significant correlation between mean and variance. The most suitable transformations appeared to be either logarithmic or angular—the latter being probably the more useful. The latter, moreover, tended to increase the sensitivity of analysis. The importance of residual counts when hatching is complete is emphasized even in the absence of an angular transformation of the data.

It is considered that for general purposes single cyst hatching is not to be recommended in view of the great expenditure of time. It is suggested that hatching of bulk samples of cysts would be more profitable, the size of a bulk sample to be as large as possible. The author favours one hundred as a reasonable compromise between accuracy and convenience.

Acknowledgments.

The author is indebted to Mr. G. V. Dyke for many suggestions and advice on the handling of the data. He also wishes to thank Miss Elizabeth Reid, who was responsible for the collection of the root diffusate used in these experiments and who also played a large part in setting up and examining the single cyst hatchings described.

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The Control of *Anguillulina dipsaci* on the seed of Teazle and Red Clover by Fumigation with Methyl Bromide.

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The stem eelworm *Anguillulina dipsaci* (Kühn) Gerv. & v. Ben., is responsible for disease in numerous plants, amongst which are teazle, *Dipsacus fullonum* L., the type host and red clover, *Trifolium pratense* L. In both cases seed harvested from infected plants will often be infected by the stem eelworm in spite of the greatest care in dressing the seed.

Kühn (1857) in describing the parasite for the first time said :— The seeds of the diseased teazles differ from those of the healthy ones. They are not half so large nor so sharply angular. Whilst the pappus of the healthy seed is stalked that of the diseased seed is sessile and almost twice as large. The worms do not completely fill the diseased seed but occur within the atrophied ovule and also as small clumps of worms within the tissue of the abnormally thickened seed coat. The worms are found not only within the diseased seeds but in all other parts of the head including the pith where they cause not only abnormal growth but death and brown discolouration of the tissues.

The author has recently seen the same set of symptoms in teazle material kindly sent to this department by Miss E. L. Britton and Mr. L. N. Staniland, South Western Province, National Agricultural Advisory Service, and there is little or nothing to add to Kühn's admirable description.

A search through the records fails to find any mention of the stem eelworm on teazle in this country. In a recent letter to the author, Staniland reports that he and Miss Britton discovered the disease early in 1948 in Somerset, the main area for teazle growing in this country.

The teazle is a biennial and in the spring of the second year diseased plants are stunted and have a " cabbagy " appearance. In the same letter Staniland also reports that he saw " cabbagy " plants in 1931. These too were from Somerset and though he did not then look for *A. dipsaci*, the symptoms were entirely typical and almost certainly the stem eelworm was the pathogen.

The first record of *A. dipsaci* on red clover seed seems to have been made by Cobb (1924) in a brief note presented to the Helminthological Society of Washington. He soaked out live worms from seed which had been cleaned and recleaned three times. Later (1929) he reported finding " these nemas adhering in a desiccated but living condition to the surface of the seeds."

In 1944 T. Goodey (in litt.) examined 504 samples of red clover seed supplied to him by the Official Seed Testing Station, Cambridge. From 32 samples he was able to soak out specimens of *A. dipsaci*, which in all cases were pre-adult larvae. Of these infected samples about a third yielded live worms so that these particular samples may be regarded as potential sources of further outbreaks of the disease.

The author has examined diseased florets of red clover and has found numerous worms within the calyx tube and within the corolla up to the level of the calyx mouth. Worms have also been found within the tissues below the various flower parts and in some cases worms had caused abnormalities in the ovary. Failure of the latter to develop properly and the production of stunted styles were the most usual changes ; this was coupled with the occasional presence of worms within the ovary and the absence of ovules.

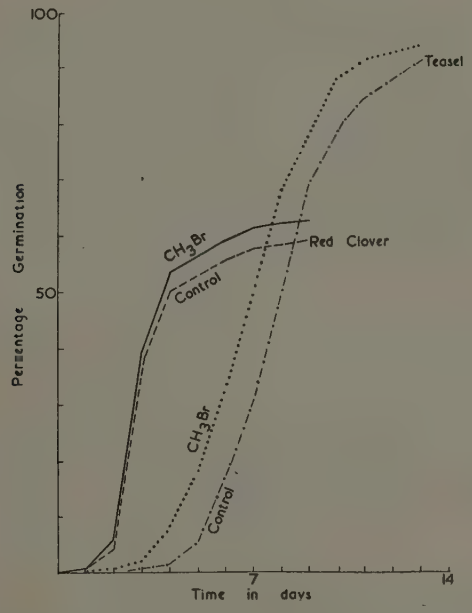
The cleaning of small seeds is never an easy matter and even after most careful dressing there is often debris left in the final product. Teazle must be extremely difficult to clean as the seeds and other parts of the heads are covered with short hairs tending to encourage adhesion of debris. Indeed, it seems that it is customary, after the heads have been disposed of, to sweep up the seed from the floor of the barn, roughly sieve it and plant the remainder including a large amount of plant tissue. There can be no doubt that the disease is further spread by these means.

T. Goodey (1943) observed that onion seed which had been most expertly cleaned still had *A. dipsaci* in a living condition adhering to the seed coats. Although Cobb (1929) reported that treatment of red clover seed with warm water at 118°F. for 15 minutes killed the parasite and left the seed uninjured, T. Goodey (1945) was unable to repeat this successfully with onion seed, even though he treated it for 30 minutes at 118°F., that is twice the time. Apart from this failure to reproduce Cobb's results with warm water a further objection is the necessity for rapid drying of the seed after treatment and the probability that it should be sown almost immediately.

He devised therefore the method of fumigation with methyl bromide. He showed that by adopting a minimum time-concentration

product of 600 and arranging that fumigation should proceed for at least 18 hours, *A. dipsaci* in the desiccated, resistant state, adhering to the seed coats was destroyed. He reported also that apart from a slight retardation in germination rate, there were no deleterious effects to the onion seed.

The author has recently fumigated infected seeds of both teazle and red clover with very satisfactory results. Treatment was arranged



to give a standard time-concentration product of 600 and fumigation was carried out for 20 hours, using an 800 c.c. glass stoppered bottle as the fumigation chamber. No living worms could be obtained from the fumigated material by soaking but were numerous from the control lots of seed. The accompanying graph shows the effect of fumigation on the germination of the seeds. The red clover was not a very good sample of seed but the curves for both it and teazle indicate the similar behaviour of both untreated and treated samples. An interesting point is the slightly enhanced germination of the fumigated seed :

Cobb found a similar phenomenon with the warm water treatment of red clover seed. One very noticeable thing was the comparative absence of fungi in the Petri dishes containing the treated seeds during germination. In each case seedlings were grown on for some weeks and were perfectly normal in their growth, as indeed T. Goodey found in the case of onion seedlings.

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A Cotton Blue-Lactophenol Technique for Mounting Plant-Parasitic Nematodes.

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The lack of a rapid and reliable method of making permanent preparations of nematodes is most strongly felt in the case of the plant-parasitic forms. The method in general use for most plant- and soil-inhabiting nematodes consists in relaxing the worms by gentle heat, fixing in 5% formalin or Ditlivsen's fixative (Thorne, 1925) and then impregnating slowly with glycerine. This procedure is quite reliable for the non-plant-parasitic forms but its results with plant-parasitic worms are very uncertain. With the latter it is necessary to transfer from fixative to very dilute glycerine, usually 1.5% glycerine in 7.5% alcohol with a trace of thymol or copper sulphate to discourage moulds. The glycerine is allowed to concentrate very slowly during at least four weeks until the worms can be transferred to pure glycerine in which they are mounted. Even then the results are often disappointing for the worms very frequently collapse.

In considering this general problem recently the authors called to mind the appearance of worms stained within plant tissues by the acid fuchsin-lactophenol method (Goodey, 1937 : Franklin, 1949). This method causes no collapse or distortion of the worms and takes but a few minutes from living material to permanently stained preparations. The process has now been developed for use with free specimens of *Anguillulina dipsaci* and plant-parasitic species of *Aphelenchoides*, and is as follows :

1. Relax worms in water by gently heating.
2. Fix overnight in F.A. 4:10. (Formaldehyde 4%, acetic acid 10%).
3. Transfer to lactophenol containing 0.01% cotton blue and heat till liquid fumes, or put into the coloured lactophenol at a temperature of 60–70° C. for 2–3 minutes.
4. Mount in slightly tinted lactophenol, sealing cover-slip with Thorne's cement (" Zut ") or lactophenol gum.

1. Tease apart the infected material in tap water or extract the worms by the Baermann funnel technique or by sieving (Goodey, 1949). Transfer the worms to a small quantity of water in a deep hollow slide by means of a bamboo or hair needle. Hold this slide over a small flame till the worms are seen suddenly to straighten ; they are then relaxed.

2. Fix the worms in F.A. 4:10. This particular mixture (10cc. formalin, or 40% formaldehyde, 10cc. glacial acetic acid and 80cc. water) was arrived at in an attempt to find a fixative which would cause little or no alteration in size when used on plant-parasitic nematodes. It fixes excellently and there is an average shrinkage of about 1%, which in practice is negligible. The transfer of worms from Ditlivsen's fixative, which contains alcohol, into lactophenol always causes considerable shrinkage and distortion. When 5% formalin is used as the fixative the results are not so good as with F.A. 4:10, though no distortion occurs.

The worms are transferred from water to fixative by the bamboo or hair needle, or the water may be withdrawn by means of a very fine pipette and the worms flooded with the fixative. A much more rapid method if one is dealing with numerous worms is to centrifuge them and pour off most of the water. Then heat the tube gently until the worms relax and add an equal volume of double-strength fixative. The worms may be stored indefinitely in the fixative in small corked tubes.

3. Transfer the worms to a drop of cold lactophenol containing 0.01% cotton blue on a slide and heat gently till the liquid fumes. Or, a better method is to have the slide with a drop of the coloured lactophenol on a hot plate such as a 6-in. square sheet of 16-gauge brass heated at one corner by a small flame (see Fig. 1). The worms are then transferred from the fixative to the lactophenol and left in it for 2–3 minutes. The temperature must not be below 60° C., but may rise to 80° C. The lactophenol must be fresh as, after five minutes or so on the hot plate, according to the size of the drop, it becomes syrupy by evaporation and worms put into it are quickly distorted. At 55° C. or below the worms will collapse and fail to take up the stain ; at 60° C. they bend somewhat on entering the lactophenol but recover completely and become sufficiently stained in 3–5 minutes ; at 70° C. they recover more quickly and staining takes 2–3 minutes. At this temperature the entry of the lactophenol must obviously be very rapid.

Acid fuchsin in lactophenol was also tried but it is a little too catholic in its staining ; cotton blue is delicately selective.

Measurements of worms before and after treatment with lactophenol show that there is very little change in size, an average shrinkage of about 4–6%, whereas worms which have been processed by the concentrating glycerine method show a shrinkage up to about 11% ; this latter is for *Anguillulina dipsaci* in particular.

4. Mount in lactophenol containing .0025% of cotton blue, taking care to use just the correct quantity to fill the area to be covered by the coverslip. Arrange three short lengths of glass rod, of the same diameter as the worms, radially around them to prevent pressure by the coverslip. Warm the latter to reduce the risk of trapping air bubbles when it is lowered over the drop of lactophenol. Seal with

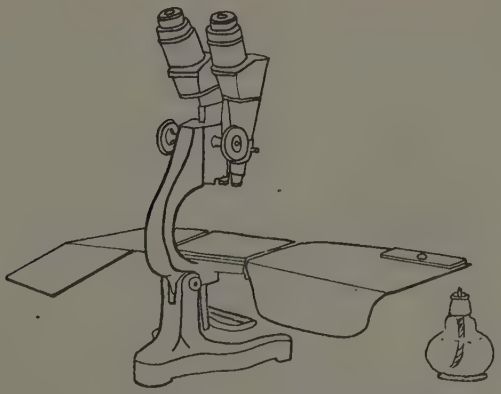

Fig. 1.—Binocular microscope with brass stage extensions: that on the right used as a hot-plate.

Thorne's cement ("Zut") (Thorne, 1935 and in litt.) or lactophenol gum (Davis, 1924). The formulae for these two sealing agents are:

Thorne's Cement ("*Zut*").	Gm.
Archer Daniels Midland 100 linseed oil (American), or Young-husband Barnes & Co. alcohol-soluble T.V. linseed oil (British)	31.75
Industrial spirit	4.76
Nitrocellulose $\frac{1}{2}$-second cotton (American), or I.C.I. nitrocellulose HX 30/50 containing 30% alcohol (British) ...	22.67
Butyl acetate	20.41
Toluol	20.41
	100.00

This is a clear varnish-like medium with which glycerine or lactophenol mounts can be ringed very satisfactorily ; it is a good cement for sticking such things as Perspex to glass, and it dries in a few minutes. It may be tinted with an oil-soluble pigment. Butyl acetate is used as the thinner.

Lactophenol Gum.

Dissolve 38 gm. of very pure gum arabic in 50 cc. of freshly distilled water ; add 5 gm. glucose, 6 gm. lactophenol and filter through glass wool. It is used cold and dries in 2–3 hours.

The authors have tried this new method on worms which had been kept in fixative but which had dried up, a reprehensible but not uncommon state of affairs. The result was surprising, for the worms, which by most standards would have been considered of little use as specimens, responded very well and made good mounts with, at most, very slight distortion. The method was also tried on specimens which had been processed to strong glycerine. Their appearance was an improvement on those mounted in glycerine, though not so good as those treated with lactophenol alone. Worms which had become shrunk through over-rapid processing in glycerine were improved when they were transferred to lactophenol with cotton blue and heated.

When the method was applied to species of *Mononchus*, *Dorylaimus* and other free-living or saprophagous nematodes, the results were extremely pleasing. *Mononchus* and *Dorylaimus* were, perhaps, a little too transparent, but there is no reason to suppose that the same method can be used with equal success for all types of nematode. The main recommendations of this method are the rapidity and certainty of the good results and the increased contrast due to the stain.

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Nutritional Factors affecting the Rate of Development of *Fasciola hepatica* in *Limnaea truncatula*.

By S. Brian Kendall, B.Sc., M.R.C.V.S., A.R.C.S.

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Factors affecting the rate of development of trematode parasites in their molluscan hosts do not appear to have been studied to any great extent although various authors from the time of Thomas (1883) have suggested that temperature has a marked effect. The effect of temperature will be discussed in a later paper but initially the effect of certain other factors is considered. Previous observations by Ross (1930) and Krull (1941) suggested that the development of trematodes was influenced by factors other than temperature, for both these authors noted that the time necessary for the full development of the cercariae of *Fasciola hepatica* showed considerable variation in individual snails infected at the same time and kept at the same temperatures.

At Weybridge, during the course of numerous observations on the liver-fluke *Fasciola hepatica* and its British vector *Limnaea truncatula* there were many opportunities for noting the number of parasites which matured in infected snails and the rate at which cercariae developed in groups of snails under different environmental conditions. It was observed that there was considerable variation in the number of cercariae which emerged from different groups of snails kept at similar temperatures but in different tanks in the laboratory. Since it appeared that large numbers of cercariae emerged most often from snails which had grown rapidly during the period after infection it seemed that the number of cercariae which matured might be related to the amount of food made available to the rediae through the tissues of the snail.

There was evidence in favour of this view, for Faust (1920), during observations on snails and their trematode parasites, found that the large amount of glycogen, fat and protein normally present in the digestive gland epithelium and intertubular sinuses of uninfected snails disappeared in cases of heavy infection and was apparently transferred to the parasites. If an extensive transference of food from the reserves of the snail to the parasite takes place without comparable replacement as the result of active feeding by the snail, the development

of the parasite may be expected to suffer. To test this assumption it was decided to compare the rate of development of the rediae of *Fasciola hepatica* in snails kept under starvation conditions and in snails having access to adequate supplies of food.

I. Relationship between Development Rate in well-fed Snails and that in starved Snails.

Material and Methods.

Snails used in the observations were from a laboratory-bred strain of *Limnaea truncatula* which had been maintained for several generations under conditions which precluded adventitious infection with *Fasciola*. In the first observation each snail was placed in a small glass tube half-filled with water to which a single active miracidium was added by means of a fine glass pipette, while in the second, the snails were exposed to infection with large numbers of miracidia.

After 24 hours, infection was assumed to have occurred and the snails were placed in dishes where they were maintained at moderate temperatures and under good environmental conditions similar to those described by Taylor and Mozley (1948) until dissection of a representative sample showed that the rediae of *Fasciola* were present in the digestive gland. The snails were then placed in small groups under the experimental conditions either with access to food, which consisted of the green algae growing in the dish, supplemented by a mixture of equal quantities of powdered chalk and oatmeal, or under starvation conditions in dishes which contained only sterilized sand and filtered pond-water. Dissection was commenced as soon as active cercariae were found to be present, paired observations from the two groups being made at intervals until all the snails had been examined.

All rediae found on dissection were compared for size and state of development but it was decided to use the total number of mature cercariae found in each snail at the time of dissection as the main criterion of development. This total was obtained by counting the number of free cercariae found in the body cavity and adding the number of mature cercariae present in the rediae which were examined separately if few in number, while if very many rediae were present the number of cercariae was estimated from the examination of random samples. Only those cercariae showing full development of the lateral cystogenous glands were classed as mature, less well-developed cercariae and undifferentiated embryos being ignored. The number of mature cercariae counted in this way represented the total number of parasites

which had matured since infection, for there was no evidence of the natural emergence of cercariae from any of the snails during the periods of observation.

Experimental Data.

Both the observations illustrated respectively in Table I and Table II compare the rates of development in starved and well-fed snails but were conducted under different conditions and at different times of the year.

Single Miracidium Infections.

Table I compares two series of snails series A and series B, each consisting of ten snails which became infected after exposure to a single miracidium of *F. hepatica*. Following infection, the snails were kept in a cool laboratory where development of the parasite proceeded slowly.

TABLE I.

Comparing shell lengths, number of rediae and the number of mature cercariae found in snails after exposure to infection with a single miracidium.

Days under experimental conditions	Series A			Series B		
	Well-fed Snails			Starved Snails		
	Shell length	Number of rediae	Mature cercariae	Shell length	Number of rediae	Mature cercariae
44	0.80 cms	39	219	0.68 cms	14	34
	0.73 ,,	22	138	0.61 ,,	17	50
	0.76 ,,	29	114	0.59 ,,	14	95
48	0.86 cms	36	339	0.71 cms	29	78
	0.69 ,,	15	188	0.60 ,,	13	106
	0.67 ,,	10	83	0.71 ,,	9	43
69	0.79 cms	17	150	0.69 cms	10	82
	0.76 ,,	41	512	0.62 ,,	11	52
	0.82 ,,	33	220	0.61 ,,	10	60
	0.70 ,,	18	9	0.63 ,,	8	114

In five weeks' time, when dissection of a small sample showed that young rediae had reached the digestive glands, groups of snails were placed under good environmental conditions in culture dishes, while others were placed under starvation conditions in dishes which contained sterilised sand and filtered pond water. The observation was carried out during the winter months and growth of the snails was not very rapid in either series. Development of the parasite proceeded, however,

and at the end of 40 days under the experimental conditions, mature cercariae had formed. Ten snails from either group were dissected, paired observations being made on the 44th, 48th and 69th days and Table I contrasts the total number of rediae and the total number of mature cercariae found in each snail together with its shell length as an index of size.

In both series only about 30% of the snails dissected were found to be infected. Our observations on the effect of exposing *L. truncatula* to infection with a single miracidium showed variable results, but failure to become infected was usually attributed to the difficulty of manipulating the exceedingly delicate miracidium.

TABLE II.

Comparing shell lengths, number of rediae and the number of fully mature cercariae found in snails after exposure to infection with large numbers of active miracidia.

Days under experimental conditions	Series A — Well-fed Snails			Series B — Starved Snails		
	Shell length	Number of rediae	Mature cercariae	Shell length	Number of rediae	Mature cercariae
32	0.96 cms	151	1353	0.62 cms	133	200
33	0.82 cms	296	1308	0.74 cms	215	218
	0.92 ,,	141	1078	0.72 ,,	169	374
35	1.02 cms	133	1299	0.67 cms	215	134
	0.82 ,,	152	952	0.74 ,,	119	391
	0.93 ,,	166	1390	0.61 ,,	159	14
	0.93 ,,	136	1160	0.68 ,,	196	9
38	1.09 cms	136	1593	0.62 cms	30	131
	1.22 ,,	344	2275	0.63 ,,	57	292
41	1.08 cms	200	2018	0.62 cms	82	480

Total number of mature cercariae from ten snails { Group A = 14,426 / Group B = 2,243

Difference 12,183

Multiple Miracidium Infections.

Table II compares two series of snails, series A and series B, each of which consists of ten snails which became infected after exposure to

large numbers of the miracidia of *F. hepatica*. After infection, all snails were kept in a cool laboratory for a period of three months by which time large numbers of rediae full of undifferentiated germ-buds were found to have developed.

The snails were then divided, as in the previous experiment, into two series, one having access to food while the other was maintained under starvation conditions. The observation was carried out during the summer months when plenty of green algal material was available as food for the snails, which grew very rapidly. After 32 days, dissection of snails from the two series showed that numerous cercariae had matured.

Table II compares shell lengths, numbers of rediae and the numbers of mature cercariae found in each snail.

It was observed that all snails in these groups had become infected as the result of exposure to large numbers of miracidia.

Conclusions.

The relative development of rediae.

It is apparent from a comparison of series A and series B in both Tables I and II that the extent to which the rediae have developed, as assessed by comparison of the numbers of mature cercariae, is related to the amount of food which has been available to the snail. During the observation, dissection showed that those snails with access to food contained much larger rediae and many more mature cercariae than those kept under starvation conditions. The effect of feeding the snails was to accelerate the rate at which the parasites matured so that in a given time many more mature cercariae were formed.

This was most clearly shown by a comparison of the total number of cercariae which matured in the two series of the second observation (Table II). In series A a total of 14,426 cercariae matured from ten snails with access to adequate food while in series B only 2,243 were formed.

A similar but less marked effect was observed with the two series of snails which had been infected with a single miracidium (Table I). The smaller difference between the two series of this group may have been caused by the winter conditions of the experiment which limited the rate of development of the series having access to food but, as will be shown later, retarded development of the parasites under starvation conditions is less marked when relatively few rediae are established in the snail and it will be seen that in most instances many more rediae

became established as a result of multiple miracidial infection than as a result of infection with a single miracidium.

The numbers of rediae found in individual snails.

Numerous observations on these and other groups of snails showed that at laboratory temperatures it was very rare to find more than forty rediae following infection with a single miracidium but that much larger numbers usually resulted from mass infection. In Table I it is shown that between eight and forty-one rediae resulted from infection with a single miracidium while Table II shows that an average of more than 150 rediae followed exposure to infection with many miracidia, as many as 344 being found in a single snail.

One snail from the group exposed to mass infection contained only 30 rediae, this probably indicating successful penetration of a single miracidium out of the large number to which the snail was exposed.

In each series there was a somewhat wide variation in the number of rediae which were found at the time of dissection, the number presumably depending firstly on the number of miracidia which became established and secondly on the number of rediae developing from each sporocyst. In addition, it will be noted that rather fewer rediae were found in the starved than in the well-fed snails, but this may be attributed to experimental error resulting from the difficulty of finding the smaller ill-developed rediae of starving snails.

Effect of the number of rediae on the rate at which cercariae mature.

It has been shown that the amount of food available to the rediae is a factor controlling the number of cercariae which mature in a given time. This food is limited by the amount ingested by the host but is related also, it is suggested, to the total number of parasites which become established in each snail. The rediae within a snail are in competition among themselves for a limited amount of food. A large number of rediae in a poorly fed host has little opportunity for growth and their germ-buds remain undeveloped, but a small number of rediae in a snail which has accumulated good reserves of food before infection may be able to produce considerable numbers of mature cercariae even if the snail is for a period kept under starvation conditions. It appears therefore, that the rate at which the parthenitae of *F. hepatica* mature is governed by two factors both related to the supply of food, the first being the actual amount of food consumed by the snail and hence the amount available from its tissues, and the second the number of parasites competing for food within the snail.

II. Relationship between Numbers of Rediae and their Rate of Development.

In the observations already described it appeared that the rate of development of rediae established in large numbers was much more likely to be adversely affected by food shortage than that of rediae established in limited numbers. To illustrate the point more clearly it was decided to compare the rate at which cercariae matured in further groups of snails in which the only variable for consideration would be the numbers of rediae.

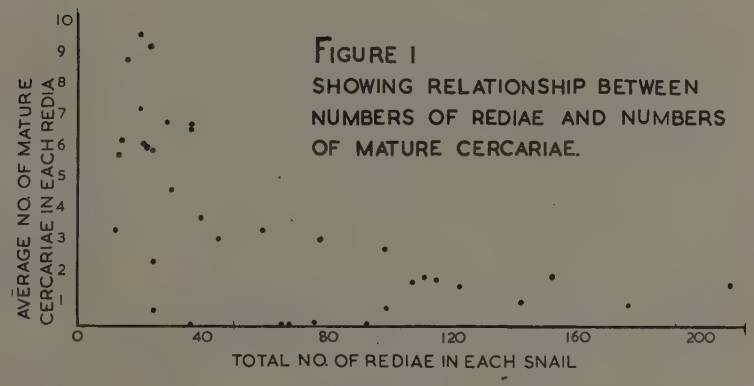

FIGURE 1
SHOWING RELATIONSHIP BETWEEN NUMBERS OF REDIAE AND NUMBERS OF MATURE CERCARIAE.

Material and Methods.

Laboratory-bred snails were infected, as in previous observations, with active miracidia of *F. hepatica*, some snails being exposed to a single miracidium while others, in groups of 20–30, were mass infected. The snails were then placed under good environmental conditions until mature cercariae had formed, when they were moved to a cold room which inhibited further development until dissection of the group was completed.

Experimental Data.

The results of these observations are shown in Table III and in Figure 1. Table III records observations on two groups, group A and group B, each consisting of seven snails. These two groups were dissected at different intervals after infection and are not comparable

one with the other. Snails within each group were dissected on the same day and the tables compare shell lengths (as an index of size and growth), the total number of rediae, and the total number of mature cercariae in each snail and as an index of the state of development of the rediae, the average number of mature cercariae per redia.

Figure 1 refers to a third group of thirty-five snails and shows the relationship between numbers of rediae and their average state of development, as indicated by the number of mature cercariae.

TABLE III.

Comparing shell lengths, number of rediae, total number of mature cercariae and the number of mature cercariae per redia.

Snails	Group A (two single and five mass infections)			
	Length	Number of rediae	Mature cercariae	Cercariae per redia
1	0.75 cms	28	701	25.0
2	0.76 ,,	37	502	13.6
3	0.62 ,,	163	166	1.0
4	0.60 ,,	168	0	0.0
5	0.70 ,,	171	191	1.1
6	0.67 ,,	208	0	0.0
7	0.80 ,,	231	795	3.4
	Group B (four single and three mass infections)			
1	0.69 cms	14	165	11.8
2	0.65 ,,	21	253	12.0
3	0.54 ,,	23	160	6.9
4	0.79 ,,	28	228	8.1
5	0.58 ,,	73	116	1.6
6	0.68 ,,	98	200	2.0
7	0.63 ,,	154	149	1.0

As in previous observations, cercariae were classed as mature if the cystogenous glands were fully developed and included both the mature cercariae still present in the rediae and those moving freely in the body cavity of the snail.

Conclusions.

As in previous observations, infection with a single miracidium resulted in the development of a limited number of rediae. In fact,

the number never exceeded 37 although very many more, as will be seen, followed mass infection.

The establishment of moderate numbers of rediae was followed by rapid growth and quick maturity, individual rediae containing as many as seventeen or eighteen mature cercariae at the time of dissection but when larger numbers of rediae became established they developed much less rapidly and contained fewer mature cercariae. This is well illustrated in Table III, group A. Snail No. 1 in which 28 rediae became established contained 701 mature cercariae at the time of dissection (an average of about 25 per redia) and in snail No. 2 an average of 13.6 cercariae matured from each of 37 rediae, but on the other hand very few cercariae matured in the rediae of the remaining snails of the group and in two which contained 168 and 208 rediae respectively, no mature cercariae at all could be found. Group B of Table III, illustrates a very similar observation on seven snails which were dissected at an earlier stage in the development of the parasite.

Figure I shows the relationship between numbers of rediae and their average stage of development (as indicated by the number of mature cercariae per redia) in a group of 35 snails and in spite of a rather wide range in values due to considerable variation in the size and state of nutrition of the snails, confirms the observation already recorded in Table III. Data are presented in the form of a graph in order to direct attention to a further aspect of the host-parasite relationship. The form of the curve, to which most of the co-ordinates correspond, suggests that the maximum number of rediae allowing rapid development to occur is about forty but that development is considerably retarded when this number is exceeded. Consideration of this critical number of rediae is important since it has already been noted that infection with a single miracidium seems to lead, at laboratory temperatures, to the development of not more than forty rediae. It appears, therefore, that the economy of the snail is adapted to infection with a single miracidium. Parasites resulting from infection with a single miracidium mature rapidly while mass infection, leading to the establishment of rediae in numbers greater than the economy of the snail can sustain, results in retarded development.

Discussion.

From the observations described in this paper it appears that the rate at which cercariae mature is related to the amount of food available, this in turn depending on the amount of food assimilated by the host and also on the number of rediae which are in competition for food.

Host-parasite relationships of this kind do not appear to have been recorded very frequently. Ackert *et al* (1940) showed that the worm *Ascaridia lineata* thrived much better in normally-fed chickens than in those fed only by glucose injections but this is perhaps not a closely parallel observation since it appears that *A. lineata* feeds directly on the ingesta of the host. Perhaps a closer analogy is derived from a study of the host parasite relationships of some insects. Daniel (1932) mentioned that the rate of development of the egg of *Macrocentrus ancylivorus* is influenced considerably by the condition of the host larva in which it has been deposited. Development follows more rapidly when the egg is laid in the later instars of the host and Daniel suggests that this is associated with the presence of larger fat bodies.

The effect of food on the rate at which cercariae mature does not appear to have been considered to any great extent in the extensive literature dealing with trematodes although Brackett (1940) mentions that feeding snails often caused a resumption of the emergence of schistosome cercariae. Many workers, after observing the intermittent emergence of trematode cercariae from their hosts have attempted to deduce a rhythm of development. In many instances, observations were carried out on snails which were removed from culture dishes where food was available to clean vessels where the emergence of cercariae could be more readily observed. It seems in such instances that the periodicity of emergence was more likely to have been related to the availability of the host's food than to a natural rhythm of development. At Weybridge it has long been customary, in order to obtain maximum numbers of cercariae of *F. hepatica* for experimental work, to return snails to their feeding-dishes after each short period during which cercariae have been induced to emerge and Bauman *et al* (1948) have described a similar procedure which they adopted to obtain large numbers of schistosome cercariae from the snail *Oncomelania quadrasi*.

Our observations have failed to show that there is any fundamental rhythm affecting *F. hepatica*, the rate of development being controlled principally by temperature (to be discussed in a later paper) and by the nutrition of the host.

In the field, the fact that the development of *F. hepatica* is retarded during periods of food shortage is of vital importance to both host and parasite, particularly in view of the periods of drought-induced dormancy which appear to be so characteristic of *L. truncatula* and during which the snail is unable to feed. Under such conditions, the premature death of host and parasite might be expected were it not for the existence of a control over the rate at which the parasites develop and a limitation of their demands during periods of food shortage.

Summary.

1. This paper describes some hitherto unrecorded aspects of trematode development in a molluscan host.

2. It is shown that the rate of development of *Fasciola hepatica* in its host *Limnaea truncatula* is influenced not only by temperature but by the amount of food assimilated by the snail and by the number of rediae which are in competition within a single host.

3. The most rapid development of the parasite will occur in hosts which have access to ample supplies of food and in which moderate numbers of rediae become established. At laboratory temperatures, infection with a single miracidium rarely leads to the establishment of more than 40 rediae, a number which is apparently adjusted to the economy of the host, since it allows development at a maximum rate.

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